ASPECTS OF THE ECO-PHYSIOLOGY OF THE FRESHWATER CRAYFISH, *Parastacoides tasmanicus* (Clark 1936)

by

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ABSTRACT

Parastacoides tasmanicus is a burrowing crayfish found on button grass plains in western Tasmania. While this project was in progress, the taxonomy of the genus *Parastacoides* was revised, and one species, *P. tasmanicus*, with three sub-species, *P. tasmanicus inermis*, *P. tasmanicus tasmanicus* and *P. tasmanicus insignis*, was recognized. *P. tasmanicus inermis* lives in drier areas than the other two subspecies and was not present at the site near Scott's Peak Dam where animals for this study were collected. *P. tasmanicus insignis* and *P. tasmanicus tasmanicus*, which have largely overlapping habitats, were both found to be present, but although they are considered to be subspecies, they differ only slightly morphologically, and physiologically, and so were not distinguished between. Except where it is otherwise stated, references to *P. tasmanicus* refer to both of these subspecies.

Measurements of a number of environmental factors over the course of the study showed that *P. tasmanicus* at Scott's Peak Dam are exposed to low pH (3.7 - 5.6), low oxygen levels (at least as low at 0.86 mL/L), and periods without free water in their burrows, although it is unlikely that the relative humidity at the bottom of the burrows would fall much below 100%, as even just inside the mouth of 'dry' burrows the relative humidity is 80% or more. Surface temperatures during the study ranged between -3°C and 39°C, but at depths of 40cm the yearly temperature range was probably not much more than 3° to 16°C, the range of burrow temperatures on collecting trips.

Almost 600 animals were collected at the Scott's Peak Dam site, 427 of which were over 1g weight. The weight-frequency distribution of these animals showed no detectable year classes, but there was a distinct difference in the weight-frequency

distribution of males and females. Males appear to grow at a steady rate throughout their life, while the growth of females slows dramatically when a weight of approximately 3 g is reached. Females become sexually mature at this size, and 50% of the females over this weight are in berry or are carrying hatched young from the end of May until the end of January. No females are in berry in March.

P. tasmanicus is very tolerant of a wide range of pH. Oxygen consumption was found to be unaffected by pH in the range of approximately pH 2.7 to 10.0 at 15°C, and pH 2.7 - 7.6 at 5°C, whilst haemolymph pH was only slightly affected by an external pH range of approximately pH 3 - 11 during a 110 hour exposure period. When *P. tasmanicus* was exposed to pH 2.5 there was a large loss of sodium ions to the external medium, although no loss of potassium was observed. At a pH of 4.8 the loss of both of these ions was negligible. It was concluded that whilst the tolerance to both high and low pH by this crayfish is quite remarkable, the causes of death at both very high and very low pH do not appear to be different from those of animals less tolerant to extremes of pH.

When kept out of water at high humidities and at temperatures of 15° and 20°C, *Parastacoides* was found to have a low rate of water loss and a high lethal water loss compared to other crustaceans. Both adults and juveniles can survive indefinitely out of water at 100% relative humidity, if they have access to damp filter paper, but they are not able to moult successfully. Large animals without access to free water survived at 100% relative humidity for up to 7½ weeks, at 15°C, while smaller animals

survived for shorter periods. At lower relative humidities survival time is reduced, but survival times for *P. tasmanicus* are higher than those of most other semi-terrestrial and terrestrial decapods. The water lost by *P. tasmanicus* in humid conditions is almost entirely lost via the gill chambers, with negligible water loss via the integument.

Parastacoides tasmanicus normally feeds on fresh or decomposing plant material, although animal food is taken when available. A study of the digestive enzymes of the crayfish, at test temperatures considerably above environmental temperatures, and at the optimal pH of the enzymes concerned, demonstrated a moderate lipase/strong esterase, strong protease, amylase and cellulase activities, and weak 'native cellulase' and chitinase activities. The lower activities observed at environmental temperatures would be counteracted by the slow passage of food through the gut, since rate of passage decreases as temperature decreases. Measurements of assimilation efficiencies of *P. tasmanicus* eating controlled diets, showed that animal food is assimilated with efficiencies of over 88%, while plant food is assimilated with an efficiency greater than 72%, at both summer and winter temperatures. When fed button grass mud, the crayfish is able to select the high-energy food components from the mud in preference to the lower-energy components and inorganic material.

A study of the metabolic activity of *P. tasmanicus* (as measured by the rate of oxygen consumption), showed that the crayfish has a lower oxygen consumption, at normal environmental temperatures, than other decapod crustaceans. It shows very little in the way of compensation for seasonal temperature changes, and so its oxygen consumption exhibits a yearly cycle, with a maximum in February (for 1 g animals) or March (for animals of 5 g weight

or over) and a minimum in August. The maximum rate is 2 to 4 times the minimum rate, with seasonal temperature changes affecting the respiration of smaller animals more than that of larger animals. Animals moult in summer and this is accompanied by an increase in oxygen consumption, with a maximum rate in early post-moult.

Annual variation in the organic composition of the major tissues of male, juvenile and berried and non-berried females was measured. These measurements showed that females only breed once every two years, and exhibit a two year berried - non-berried cycle. In addition, the moult at the end of the 'berried' part of the cycle probably does not involve any increase in size. During the non-berried part of the cycle, mainly during the warmer months, energy stores in the midgut gland, in the form of lipids, increase in preparation for a 'growth' moult. At the same time, the gonads are increasing in size and stage of development, so that moulting can be rapidly followed by egg production. During the berried part of the cycle the energy stores in the midgut gland and other tissues remain low, and the gonads do not grow very large.

Adult males moult once a year like the females, but every moult is a 'growth' moult. The males have a body composition similar to that of berried females.

The eggs of *P. tasmanicus* are larger and have larger energy stores than the eggs of most other decapods. The relevance of this to the reproductive strategy of *P. tasmanicus*, and the survival strategy of juvenile animals, is discussed. It is concluded that there is only a small recruitment of juveniles into the population each year, and successful juveniles will be those that are a large size at birth, and grow rapidly so that they can find a burrow and defend it against other juveniles. It is estimated that crayfish live about 8 years.

A study of the energy content of the tissues of *P. tasmanicus* supported the conclusions reached from body composition data. The energy content of *P. tasmanicus* is similar to that of benthic malacostracans; any differences can probably be attributed to incorrect techniques used by other researchers.

Parastacoides tasmanicus exhibits a unique set of responses to low oxygen conditions. When exposed to oxygen concentrations of 0.8 mL/L at 17°C, or to lower oxygen levels at lower temperatures, *P. tasmanicus* may leave the water to respire in air. The crayfish is capable of regulating its oxygen consumption down to approximately 4 mL O_2/L at both 5° and 15°C. Below this oxygen level, oxygen consumption decreases with decreasing oxygen tension. This is not a particularly low incipient limiting tension, but the important point is that the incipient lethal tension is low. *Parastacoides tasmanicus* can reduce its activity to reduce its oxygen demand, and respires anaerobically if oxygen levels are low enough. It does not have a very high tolerance towards lactic acid, but as long as the oxygen levels are not too low, it is able to excrete the lactic acid that it produces. It does not pay back an oxygen debt when it is returned to aerobic conditions after a period in anaerobic conditions. This would be wasteful in some situations, but is an important part of the strategy used by *P. tasmanicus* for coping with chronic low oxygen levels.

The adaptations used by *P. tasmanicus* to cope with different aspects of its environment are interrelated, some mechanisms being used for multiple purposes, and other mechanisms affecting many aspects of the life cycle of the crayfish. These relationships are briefly considered in the final section of this thesis.

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1. GENERAL INTRODUCTION

There are over 400 recognised species of freshwater crayfish, with endemic species occurring on all continents except Africa. North America has over 250 species, while Australia and its neighbouring islands have more than 100. These 400 species comprise 2 superfamilies in the Infraorder Astacidea (Hobbs, 1974a). All Northern Hemisphere crayfish belong to the Families Astacidae and Cambaridae in the Superfamily Astacoidea, while all Southern Hemisphere crayfish form the Family Parastacidae in the Superfamily Parastacoidea. Table 1.1 gives a breakdown of the 3 families into subfamilies and genera, and also summarises the geographical regions where the genera occur, and the number of species in each genus. The table is derived from the works of Bouchard (1978), Bouchard and Hobbs (1976), Gledhill, Sutcliffe and Williams (1976), Hobbs (1974a, 1974b, 1975), Holthuis (1967) and Sumner (1978).

Crayfish may be found in a very wide range of habitats, including lakes, rivers, creeks (permanent or intermittent), ditches, subterranean caverns, as well as burrows in swamps, woodland, fields, rainforest or bogs. They are successful in waters with pH ranging from the very acidic to very alkaline.

Hobbs (1942) classified burrowing crayfish in three groups, as follows.

Primary burrowers. Those animals that are restricted to burrows.

Secondary burrowers. Those crayfish that generally occupy burrows, (especially during the breeding season), but which often wander into open water during times when the mouths of the burrows are flooded.

Tertiary burrowers. Crayfish that only burrow in periods of drought, or occasionally, but not necessarily, during the breeding season.

Table 1.1 The distribution and number of species of the freshwater crayfish.

	Occurrence	Number of species*
Infraorder Astacidea		
Superfamily Astacoidea		
Family Astacidae		
Genus *Astacus*	Middle, northern and eastern Europe	4
" *Austropotamobius*	Western and middle Europe, Britain, Ireland	2
Pacifastacus	North America	5
Family Cambaridae		
Subfamily Cambarinae		
Genus *Barbicambarus*	North America	1
" *Bouchardina*	" "	1
" *Cambarus*	" "	57
" *Fallicambarus*	" "	11
" *Faxonella*	" "	3
" *Hobbseus*	" "	5
" *Orconectes*	" "	63
" *Procambarus*	North and Middle America	122
" *Troglocambarus*	North America	1
Subfamily Cambarellinae		
Genus *Cambarellus*	North America	12
Subfamily Cambaroidinae		
Genus *Cambaroides*	Eastern Asia	4
Superfamily Parastacoidea		
Family Parastacidae		
Genus *Astacoides*	Madagascar	1
" *Astacopsis*	Tasmania (Australia)	4
" *Cherax*	Australia, New Guinea, Aru Islands, Misool	39
" *Engaeus*	Victoria, New South Wales and Tasmania (Australia)	23
" *Engaewa*	Western Australia	3
" *Euastacoides*	Queensland (Australia)	2
" *Euastacus*	Eastern states of Australia	27
" *Geocharax*	Victoria, King Island and north-west Tasmania (Australia)	2
" *Gramastacus*	Victoria (Australia)	2
" *Paranephrops*	New Zealand	2
" *Parastacoides*	Tasmania (Australia)	1
" *Parastacus*	South America	6
" *Samastacus*	South America	2
" *Tenuibranchiurus*	Queensland (Australia)	1
		406

* Not including subspecies. These numbers are approximate only, as the number of species is sure to be altered due to revision of the taxonomy of the groups, or due to the discovery of new species.

The endemic European crayfish belong to the genera *Astacus* and *Austropotamobius* in the Family Astacidae (see Table 1.1). They are found in lakes, rivers and streams, and favour oxygenated waters, preferably neutral or slightly alkaline, with calcium concentrations above 699 mmol/L (Chaisemartin, 1967). Calcium is essential in the environment of these animals as they store insufficient quantities in the gastroliths and hepatopancreas to construct a new carapace after moulting, and must make up the deficit from the water in which they live (Chaisemartin, 1964, 1965a, 1965b, 1966, 1967; Laurent, 1973). Although not renowned as burrowers, at least one of the European crayfish can burrow to a limited extent. Thus Laurent (1973) found that *Austropotamobius pallipes* caught in a drying stream dug shallow burrows in the clay banks and bottom of the stream. *Astacus* and *Austropotamobius* species do not have any adaptations to a burrowing life and could at best be described as tertiary burrowers.

The crayfish of North and Middle America can be placed in two families and 11 genera (see Table 1.1). A summary of the number of primary, secondary and tertiary burrowers in these eleven genera is provided in Table 1.2. The data for this table comes mainly from Hobbs (1974b) with some additional data from Bouchard (1978), Bouchard and Hobbs (1976) and Hobbs (1975).

All of the *Fallicambarus* species are primary or secondary burrowers in or near lentic or lotic habitats, usually of a temporary nature, or in pine flatwoods (Hobbs, 1975). They exhibit modifications, to various degrees, that make them well adapted to their burrowing existence e.g. broad, flattened digging chelae, narrow areola and vaulted carapace, creating an enlarged branchial chamber, reduction of the tail fan and powerful abdominal muscles, deepened sternum that better encloses the reproductive structures, smaller eyes, and reduction of spination or projecting structures such as the antennal

Table 1.2 The burrowing crayfish of North and Middle America

	Primary	Secondary	Tertiary	Total number of species
Family Astacidae				
Genus *Pacifastacus*	0	0	0	5
Family Cambaridae				
Subfamily Cambarinae				
Genus *Barbicambarus*	0	0	0	1
" *Bouchardina*	0	0	0	1
" *Cambarus*	9-10	5-6	0-1	57
" *Fallicambarus*	8-9	2-3	0	11
" *Faxonella*	0	0	1-2	3
" *Hobbseus*	0	3	0	5
" *Orconectes*	0	0	1-3	63
" *Procambarus*	7	19-21	12-15	122
" *Troglocambarus*	0	0	0	1
Subfamily Cambarellinae				
Genus *Cambarellus*	0	0	2-3	12

scale (Bouchard, 1978). *Cambarus*, *Procambarus* and *Hobbseus* also contain a number of species that are primary and/or secondary burrowers, while crayfish from the other genera will only burrow when surface water disappears from their habitat, if at all. *Orconectes* would appear to be restricted to permanent water bodies (mainly creeks and streams) as Hobbs does not regard any of the *Orconectes* species as primary or secondary burrowers, and only a few may be regarded as tertiary burrowers. However, Crocker and Barr (1968) reported that *O. immunis* constructed burrows over 60 centimetres deep in hard clay soils, so this species at least might be capable of acting as a primary or secondary burrower. *Orconectes nais* has been found to dig burrows in which it spends the winter (Armitage, Buikema and Willems, 1972; Williams and Leonard, 1952) and may be regarded as a secondary burrower.

Very little non-taxonomic work has been done on North American primary burrowers, but the life cycle of some secondary

burrowers has been studied, and this may provide an insight into the life of primary burrowers. There is considerable variation between genera and also between species that are subject to different environmental regimes, but there are a number of similarities too, especially among the crayfish that live in higher latitudes (Crocker and Barr, 1968; Harris, 1903; Tack, 1941; Tarr, 1884; Williams, Williams and Hynes, 1974). These crayfish spend winter in burrows, usually in a torpid condition. In colder areas this is probably simply a response to very low environmental temperatures, that also ensures that the crayfish do not freeze to death in a shallow pond or stream. However, in cases where freezing is not likely, it may be that animals desert a stream during winter because the current is too strong for them to be able to maintain station (Bovbjerg, 1952).

In early spring, adult males and immature males and females re-enter the stream or pond near which their burrows are located, although berried females usually remain in, or at least near, their burrows. In some species the females have carried eggs over winter, and these hatch in late spring and the juveniles leave the females about a week later. In other species oviposition may occur in early or mid-spring (although mating can have occurred some time earlier), and in these cases the eggs develop and hatch very quickly, and the young normally leave the females by late spring or early summer.

During spring and summer juveniles grow rapidly and moult frequently. Males and immature females moult in early spring and again in mid-summer. The mature females do not moult until the young have left them, which means that they may only moult once during the year.

In burrowing species that live in permanent water bodies, the adults and juveniles remain in the water until towards the end of autumn. The adults may mate from mid-July onwards, with the females coming into berry as early as late October, or mating and/or spawning may be delayed until the following spring. In late autumn the animals start the return to their burrows for winter.

Species that live in water bodies that dry up during late summer spend only a brief period out of their burrows. As the stream or pond that they are in dries up, adult animals return to their burrows and deepen them as necessary to remain in contact with the dropping water table. Juveniles have to dig into the drying stream- or pond-bed or perish. As the water table continues to drop some animals may plug up their burrow entrance to reduce water loss. The crayfish remain in their burrows until the following spring.

Primary burrowing crayfish may be expected to have a similar timing of events, except that they do not spend any part of the year in a creek, pond or other water source. Presumably they are active when in the burrow, except possibly during winter.

Except for the morphological work on *Fallicambarus* referred to earlier, little investigation has been carried out to determine what adaptations have been developed by crayfish for a burrowing habit. This is somewhat surprising, despite the difficulty in obtaining specimens. However, some comparative work has been done, where stream crayfish have been compared to pond crayfish, and the results provide an insight into adaptations that a burrowing crayfish may possess.

The two crayfish, *Orconectes virilis* and *O. immunis* have similar ranges but are ecologically isolated within these ranges; the former species inhabits streams and lake margins while the latter

inhabits ponds and sloughs. Field and laboratory data suggest that *O. virilis* is excluded from ponds by its inability to survive periods of low oxygen tension as well as *O. immunis*, and by its inability to dig burrows to escape desiccation when the ponds dry up in summer (Bovbjerg, 1970).

Orconectes immunis and *O. nais* (an inhabitant of small flowing streams) have similar rates of oxygen consumption under moderate temperature and oxygen concentration conditions. However, under stresses of high temperature and low oxygen concentration, their metabolic rates differ significantly, with *O. immunis* regulating its oxygen consumption much better than *O. nais* (Wiens and Armitage, 1961).

Some other investigations have also shown that crayfish that normally live in an environment where oxygen is sometimes in short supply can survive oxygen shortages better than crayfish that always live in well oxygenated conditions (Abbot, 1873; Burbanck, Edwards and Burbanck, 1948; Larimer and Gold, 1961; Moshiri *et al.*, 1970; Park, 1945; Park, Gregg and Lutherman, 1940). Pond (and burrowing) crayfish also survive other environmental stresses better than stream crayfish e.g. high temperatures, extremes of pH and lack of water(Bovbjerg, 1952; Park *et al.*, 1940).

Another adaptation to a troglobitic or burrowing habit could be a reduction in eye size or function (Hobbs, Hobbs and Daniel, 1977). *Cambarus (Jugicambarus) nodosus*, a burrowing crayfish which builds complex burrows has eyes of a reduced size and with relatively narrow areola (Bouchard and Hobbs, 1976) as have the *Fallicambarus* species mentioned earlier. What advantage this is to the animals is not quite clear.

Other possible adaptations of crayfish to a burrowing habit, suggested by Hobbs *et al* (1977) from a consideration of Northern

Hemisphere forms are

(a) a lower metabolic rate than epigean species,

(b) a reduction in body size (small animals are energetically more efficient at digging burrows than larger animals; they can survive in small pockets of water that would be insufficient for larger animals during dry conditions; they could build small diameter burrows that would make them inaccessible to small mammalian predators, to name just a few of the advantages),

(c) the production of fewer, but larger eggs, and

(d) the utilisation of a less specialised diet.

The parastacid crayfish of the Southern Hemisphere are less well known than the astacid and cambarid crayfish of the Northern Hemisphere, but limited information is available concerning their habitats. For example, the only data on the habitats of the four subspecies of the species *Astacoides madagascarensis* from Madagascar states that two of the subspecies were collected from rivers and streams, while the other two were collected from marshes (Holthuis, 1964). It seems likely that in terms of Hobb's (1942) classification the two marsh dwelling subspecies, *Astacoides madagascarensis madagascarensis* and *A. madagascarensis granulimanus* are primary or secondary burrowers. Riek (1972) however, classifies the whole genus as 'moderate' burrowers.

The parastacid crayfish of South America belong to the genera *Parastacus* and *Samastacus*. Riek (1971) states that "*Parastacus* embraces the burrowing species with the chelae moving vertically whereas the stream and lake inhabiting species with the chelae moving horizontally are referred to the new genus *Samastacus*." In a later publication, Riek (1972) refers to *Parastacus* species as being strong (i.e. primary?) burrowers and *Samastacus* species

as being moderate (i.e. secondary or tertiary ?) burrowers. Holthuis (1952) reported that although *Parastacus pugnax* lives in holes in moors and erects a kind of chimney around the entrance, and is therefore presumably a primary burrower, *P. spinifrons* lives in water, and it must therefore be regarded as at best a tertiary burrower.

More information is available for the New Zealand genus *Paranephrops*. Hopkins (1970) described the habitats of both species of *Paranephrops* and studied the breeding habits of *P. planifrons* (Hopkins, 1967). *Paronephrops zealandicus* is found in streams, both muddy and gravel bedded, small ponds and lakes. Where the bed or the banks are suitable, it burrows, and therefore may be regarded as a tertiary burrower. *Paranephrops planifrons* is found in lakes, ponds, large and small running waters, on gravel substrates and in swamps. It has even been reported from pakihi bogs (Eldon, 1968). Eldon describes these bogs as "areas of flat land exceedingly low in plant nutrients, low in clay content and very acid". He continues: "The pakihi abounded with burrows, rather like giant worm holes, some of which went straight down into the liquid mud beneath, and some of which ran off at an angle under clumps of vegetation. *P. planifrons* was abundant both in burrows in the open and in dry water channels." *Paranephrops planifrons* obviously can be, but is not always, a primary burrower. What adaptations it has for surviving in such a hostile environment as the pakihi bogs are not known.

Australia is the stronghold of the Parastacidae, and possesses 10 genera with over 100 species (Hobbs, 1974a; Riek, 1969, 1972; Sumner, 1978). Riek (1972) segregates the Parastacidae into "two more or less distinct ecological groups with which are correlated some morphological attributes. Those species that are relatively

moderate burrowers, living mainly in permanent waters, hold the great chelae (first pereiopods) and move the fingers, either in an oblique plane or a more or less horizontal one, whereas the species that are strong burrowers hold the great chelae, and move the fingers, in a vertical plane."

Riek includes in his group of strong burrowers the genera *Engaeus*, *Engaewa* and *Tenuibranchiurus* from Australia, and the South American genus *Parastacus*, which has already been discussed. These crayfish are mostly small, and have an increased gastric mill size, probably correlated with a diet consisting mainly of plant roots. The three Australian genera could all definitely be classed as primary burrowers. As Clark (1936a) put it "Ortmann's method of collecting specimens, by digging them out with a bayonet, is not much use when dealing with the Victorian species of *Engaeus;* a pick and shovel are the best collecting instruments as many of the burrows descend six of seven feet".

Riek's group of moderate burrowers consist of the genera *Euastacus* *Euastacoides*, *Cherax*, *Parastacoides*, *Gramastacus*, *Geocharax*, and *Astacopsis* from Australia, and *Astacoides*, *Samastacus* and *Paranephrops*, which have already been discussed. People who have had to collect specimens of *Geocharax* and *Parastacoides* in Tasmania would disagree strongly with Riek's description of these animals as moderate burrowers (A.M.M. Richardson, M. Ritchie, R. Swain pers. comm, personal experience). They are strong burrowers, and build extensive and often deep burrow complexes. *Geocharax* has been known to dig burrows over 2.4 metres deep. Both *Geocharax* and *Parastacoides* can be primary burrowers, although *Geocharax* often acts as a secondary burrower, and specimens of *Parastacoides* have on occasions been found under stones in creeks. This throws great doubt on Riek's assertions about the relevance of how the two 'groups' hold the chelae, as both *Geocharax* and *Parastacoides* hold their

chelae obliquely. Some of the *Cherax* species are also known to form extensive burrows. For example, *C. punctatus* burrows down several feet to the water level and builds large cones at the entrance to its burrow (Clark, 1936a); this species is probably best regarded as a primary burrower. Other *Cherax* species are secondary or tertiary burrowers and can cause considerable damage to earthen dam walls with their burrowing. The other genera of 'moderate burrowers' are probably at best secondary, and in some cases, merely tertiary burrowers.

Descriptions of the ecology of primary burrowing parastacids have been mainly restricted to species of *Engaeus* and *Parastacoides* (Clark, 1936b; Lake and Newcombe, 1975; Riek, 1969, 1972; Suter, 1975; Suter and Richardson, 1977; Swain, Allbrook and Lake, 1977). Suter (1975) and Suter and Richardson (1977) described the burrow systems of *Engaeus fossor* and *E. cisternarius* in north-west Tasmania. Although often found in the same area, the two species are separated by habitat, *E. fossor* being found in burrows near creek beds, always in touch with the water table, whilst *E. cisternarius* occupies drier areas above the water table. Both species eat animal material (worms etc.) if it is available but plant material forms the major part of the diet. Both species are able to survive in water of low oxygen content and can also survive for a considerable time out of water, in a very humid environment. The life history and larval development of *E. cisternarius* has been described by Suter (1977).

Parastacoides tasmanicus was first described as *Astacus tasmanicus* by Erichson (1846), renamed *Astacopsis tasmanicus* by Haswell (1882) and then called *Parastacoides tasmanicus* by Clark (1936a) when she formed the genus *Parastacoides*. Riek (1967) considered that there were 6 species of *Parastacoides*, but a recent,

comprehensive study of over 300 adult specimens has brought Sumner (1978) to the conclusion that there is only one species, *P. tasmanicus*. This species comprises three sub-species, *P. tasmanicus tasmanicus*, *P. tasmanicus inermis* and *P. tasmanicus insignis*.

The distribution of *Parastacoides tasmanicus* is centred in the south-west of Tasmania, but extends over most of the western part of the island, except for the north-west corner. *Parastacoides tasmanicus* is found in a number of habitats (see Swain *et al*, 1977) but is found most abundantly on wet button grass *(Gymnoschoenus sphaerocephalus)* moors, where it digs extensive burrow systems. Newcombe (1970), Lake and Newcombe (1975) and Swain *et al* (1977) describe the ecology and life history of the species, and only the aspects relevant to the present study will be mentioned here.

In their study area Newcombe (1970) and Lake and Newcombe (1975) measured summer air temperatures which reached up to 22°C, and winter air temperatures of as low as 1°C. The mean January temperature would have been 12-14°C and the mean July temperature 4-6°C (Langford, 1965). As would have been expected, the variation in the water temperature in the burrows of *P. tasmanicus* was much less, and only ranged between 5° and 15°C during the year.

The oxygen concentration in the crayfish burrows varied considerably during the year, and in mid-summer it dropped as low as 1 mg/L. The pH of the water in the burrows had a mean of 4.4 ± 0.3. The low pH and low oxygen levels are due to the fact that there is restricted drainage on button grass plains, and this results in an accumulation of organic matter which gives very strongly acid peats (Knott and Lake, 1974), and low oxygen levels at times. The considerable tolerance of *P. tasmanicus* to a wide range of pH has been shown by Newcombe (1975).

The burrows of *P. tasmanicus* are described in detail in Lake

and Newcombe's (1975) paper and in Swain *et al* (1977). Burrows can be very extensive and normally reach down to the quartzite layer that usually underlies the organic layer on the button grass plains. Despite an annual rainfall of over 2.0 m in many parts of the species' distribution, dry periods can last for several weeks, and during these periods the water table might fall below the lowest level of the *P. tasmanicus* burrows.

Parastacoides tasmanicus females have a long breeding season. Females carry eggs over winter from April to November. The young then hatch but remain attached to the pleopods of the females until January to March of the following year, according to Lake and Newcombe (1975). Juveniles may remain in the same burrow as their mother for a while after leaving her, digging small side burrows off the parental burrow, but eventually they all have to leave and either find an unoccupied burrow or dig one of their own. Both males and females moult once a year while the juveniles moult a number of times.

The food available to *P. tasmanicus* is mainly restricted to detritus and plant material, but occasionally some animal material such as earthworms are found and eaten.

It can be seen that *P. tasmanicus* has to cope with a number of 'problems' during the year. In common with most burrowing crayfish it must survive in an environment where oxygen is often scarce. The mechanisms it uses to do this are investigated in part of this thesis (Chapter 7). The crayfish must also survive in an acid environment. *Engaeus* species (probably) and *Paranephrops planifrons* (in exceptional situations) also have to cope with this problem, but no reports of how they do so have yet been published. Chapter 3 is an investigation of the metabolic response of *P. tasmanicus* to a wide pH range, and investigates the cause of death at extremely

low pH.

As stated above, the burrows of *P. tasmanicus* can sometimes be left without any free water in them for weeks at a time, in summer. Chapter 4 investigates the ability of crayfish of various sizes to survive in conditions that would be found in 'dry' burrows, namely high humidity and high (15-20°C) temperatures.

Food is important to any animal and *Parastacoides tasmanicus* must survive on a diet composed mainly of plant materials and detritus. Chapter 5 investigates the efficiency with which crayfish digest various types of foods and examines the activity of a number of digestive enzymes, which determine what components of the diet are utilised, and how effectively they are utilised.

Female *P. tasmanicus* carry eggs and young for a considerable period of the year. These crayfish would be hard-pressed to breed each year and yet still moult and grow as well, especially as berried females are usually less active then other animals. Therefore the metabolic activity and body composition of males, berried and non-berried females and juveniles, during the year, were measured, to see how the life cycle and reproductive cycle of the crayfish are structured, and how they are associated with environmental conditions (see Chapter 6).

Finally the data from the various chapters has been integrated in an attempt to show how the mechanisms that *P. tasmanicus* uses for coping with various aspects of its environment interact, and how some mechanisms actually serve multiple purposes (Chapter 8).

2. COLLECTION AND MAINTENANCE OF *Parastacoides tasmanicus*, WITH SOME OBSERVATIONS ON ITS ECOLOGY

2.1 Introduction

As reported in Chapter 1, Newcombe (1970) and Lake and Newcombe (1975) measured some of the features of water in *Parastacoides tasmanicus* burrows at McPartlan Pass in south-west Tasmania. They measured oxygen concentration, pH and water temperatures on each of their collecting trips, and obtained data on air temperatures and rainfall from Strathgordon, the nearest town. In addition, they obtained information on the population structure, sex ratio and percentage of females in berry of the population of crayfish that they were studying. This chapter is partly concerned with measurements of the same features at a site near Scott's Peak Dam, which is also in south-west Tasmania. However, there is a difference in emphasis. Lake and Newcombe were concerned mainly with 'normal' conditions, while the emphasis here is on the 'extremes' of temperatures, pH and (low) oxygen levels that *P. tasmanicus* may have to face. Some of the later chapters are concerned with the way that the crayfish copes with these factors, so an idea of just how extreme the 'extreme' conditions can be, is required.

During the course of this three year study, a large number of crayfish were collected, and this large number of animals made it possible for some comments to be made on population structure, sex ratio and percentages of females in berry at various times of the year. Some of this data is relevant to a later chapter (Chapter 6).

In addition to the measurements mentioned above, a further piece of work was carried out. *Parastacoides tasmanicus* are solitary animals, with normally only one animal, or one female with some very small juveniles, living in a burrow. Crayfish are

very rarely observed on the surface during daylight, although they must move around above ground to some extent, at least to mate, and juveniles could be expected to spend a considerable amount of time searching for an uninhabited burrow after they have left their mother's burrow. To see which animals, if any, do move around and change burrows, a number of burrows had their occupants removed, although the burrows were left essentially intact, and these burrows were checked at a later date to see if they had been reoccupied.

2.2 Materials and methods

Specimens of *P. tasmanicus* were collected from wet button grass *(Gymnoschoenus sphaerocephalus)* plains, 2.5 kilometres south-west of Scott's Peak Dam in south-west Tasmania (43° 02' 59" South,146° 16' 24" East, 310 metres above sea level).

The soil at the collection site conformed to Nicolls and Dimock's (1965) 'moor podzol peat' and had an underlying quartzite layer 10-60 centimetres below the surface. Nicolls and Dimmock describe the origin and structure of 'moor podzol peats' as "With restricted drainage, organic matter accumulates to form shallow very strongly acid peats, overlying grey less organic sandy A horizons and a darker organic B horizon. Often this rests on clay". This area was chosen as a collection site for two reasons. Firstly, because the quartzite gravel layer was relatively close to the surface, the crayfish could not dig burrows as deep as in other areas. This meant that even on the wettest days the deepest parts of the burrows were still within arm's length of the soil and water surface, and also that burrow excavation could be carried out by one man. Secondly, the burrows were not as extensive as in other areas, so less time was required to dig up each burrow, and the chance of finding the animal in an occupied burrow, was increased. The main disadvantage of the site was that both *P. tasmanicus tasmanicus* and *P. tasmanicus insignis* were collected from it. When this study started the status of the genus *Parastacoides* was unclear. By the time Sumner (1978) showed that, on the basis of a single morphological feature there were three subspecies, namely *P. tasmanicus tasmanicus, P. tasmanicus insignis* and *P. tasmanicus inermis,* the experimental part of the research was effectively completed. It was decided that the two subspecies present at the collection site, namely *P. tasmanicus tasmanicus* and *P. tasmanicus insignis* should be treated as one because investigation following publication of Sumner's revision showed that at the collection site the two subspecies were interspersed without any readily observable pattern, and it was considered that if there were physiological

differences between the two subspecies, in the area concerned, then some separation of habitat should have been apparent. Subsequent work in the Olga River Valley (Richardson and Swain, in press) has indicated that differences do exist in the microhabitats occupied by the two subspecies. In the Olga Valley,where the subspecies occur together, *P. tasmanicus insignis* predominates in the drier areas, with the proportion of *P. tasmanicus tasmanicus* increasing as the soil becomes progressively more waterlogged. However, habitat separation between these two subspecies is much less than that between either of them and *P. tasmanicus inermis*, which inhabits much drier areas than either of the other two subspecies. In retrospect it was fortunate that *P. tasmanicus inermis* was not present in the study area, although it was found nearby.

Since Richardson and Swain showed that *P. tasmanicus insignis* inhabits slightly drier burrows than *P. tasmanicus tasmanicus* it is probable that a preponderance of the animals collected at the study site were *P. tasmanicus insignis*, as it was easier to dig up the drier burrows (especially in wet periods). However, Richardson and Swain's study area was of a uniform slope where changes in drainage characteristics and other factors were fairly easily distinguished, and even under these conditions *P. tasmanicus tasmanicus* and *P. tasmancis insignis* showed considerable overlap at the lower reaches of this slope. The overlap in habitat is likely to be even more pronounced in a homogeneous habitat like the study area. If there are very small differences in habitat requirements then it is considered likely that only minor differences in physiology exist. The search for these differences would provide a course for further study, but it is likely to involve more subtle differences than those looked for in this study, which was really involved in establishing baselines.

Of course, even if it were possible to retrospectively associate the identity of individual animals with the experimental data obtained from them this would not have benefitted this study very much; in fact it would simply have further exacerbated the problem of small numbers of animals available for experiments.

Two maximum-minimum thermometers were kept at the site. One of these measured soil surface temperature (not air temperature), while the other one was almost completely buried in the soil, and measured the soil/water temperature at a depth of 10-15 centimetres. The maximum and minimum temperatures for the periods between collecting trips were read from these thermometers. In addition, a Grant multi-probe temperature recorder was taken to the site in February 1976 to measure diurnal variations in surface and soil temperatures, for a period of three weeks. Probes were placed in the soil at various depths, ranging from just at the surface to a depth of 30 centimetres, and the plot from the Grant recorder was later analyzed to determine the daily maxima and minima temperatures at the different depths.

The oxygen concentration in burrow water was measured on occasions by the Fox and Wingfield (1938) micro-Winkler technique. On many occasions it snowed or rained heavily before and during a collecting trip, which made the measurement of oxygen concentration pointless, as the rain or snow would have raised the oxygen concentration of the burrow water to far above normal levels. On a few other occasions, especially after a warm dry spell in summer, it was impossible to find enough clean water to use in oxygen measurements. The measurements of oxygen concentration in burrow water were rough estimates only, and no modifications were used to reduce interference from dissolved organic matter. In laboratory measurements of oxygen concentrations however, precautions were taken to remove organic matter before measurements were made (see Section 3.2.1).

pH was measured with a Titron portable pH meter, or else water samples were taken in polythene bottles and the pH of these samples was measured with a Townson digital pH meter in the laboratory, within three hours of the samples being collected. Here too, measurements could not always be made in summer due to lack of water.

No measurements of the depth of water in burrows was made, but when a number of burrows were found to have no free water in them,

the date was noted.

Measurements of the ionic composition of burrow water were not made, as this study was not concerned with these, and it was assumed that because of the structure of the soil, the high rainfall and the low pH, the water would have the depauperate ion concentration typical of this type of region (Altman and Dittmer, 1966; Buckney and Tyler, 1973a, 1973b; Lake and Newcombe, 1975).

Animals were collected on the following dates:

1975 March 26
May 28
August 9
October 24
December 1
1976 February 17
March 30
May 30
August 13
November 23
1977 January 26
March 15
April 26
June 8
August 16
October 18
December 6
1978 January 9
February 7
March 7

One trip in July 1976 was abandoned because snow made the roads impassable.

Animals were normally collected between 7 a.m. and 11 a.m. by the simple but tiring method of digging up burrow systems and searching the tunnels until the occupant (rarely occupants) was (were) found. Each animal was placed into an individual '3 pint' plastic ice-cream container with approximately 500 mL of button grass water. The compact nature of the soil meant that soil could be dug up in 'slices' which did not collapse. After animals had been removed from burrows these slices could be replaced like a jigsaw, so that the burrow systems were left essentially intact. These burrows could then be easily dug up again on later

trips to check for the presence of new occupants.

The crayfish were returned to the laboratory in the ice-cream containers, dried on paper towelling and weighed. Animals under 1 g weight were called juveniles, and were considered as one group, as it was very difficult to sex live animals of this size. Animals over 1 g weight were loosely termed adults, although many of these animals were not sexually mature. The sex of these larger animals could usually be determined. Apart from weight, the carapace length of a number of animals (measured from the tip of the rostrum to the rear of the midline of the carapace) was also measured. This was done to enable a relationship between carapace length and weight to be determined, so that the results of this thesis, which refers to animal size in terms of weight, could be compared with other people's work in which size is measured in terms of carapace length.

Animals were maintained in the laboratory in the ice-cream containers, which contained water and some button grass mud. No aeration of the containers was necessary. The containers were kept in either a 5^{o} or a $15^{o}C$ constant temperature room, the selection usually being based on the water temperature on the day of capture. The crayfish were fed once every two to three weeks on commercial fish food pellets or pieces of worm. Under these conditions some animals survived for several years, with animals at the higher temperature occasionally moulting successfully.

To examine the rate at which empty burrows were reoccupied, the following procedure was followed. On April 26, 1977, two dozen crayfish burrows, which had had their occupants removed, were carefully replaced as they had been found. The burrows were marked with short stakes so that they could be readily identified and they were dug up again on June 8. This procedure enabled

recolonisation during late autumn - early winter to be assessed. The process was repeated in late winter - early spring, when 14 burrows had their occupants removed on August 16, and were dug up again on October 18 to see if they had been reoccupied by other crayfish.

Mid-February was very dry, and by the 23rd there were a number of crayfish burrows without any standing water in them, even in the bottom retreat chamber. On this day a digital thermo-humidity meter (with an HMP11 probe made by the Vaisala Co.), powered by a portable generator, was used to measure the humidity inside the tunnels of 'dry' *P. tasmanicus* burrows.

Rainfall data for the nearest town to Scott's Peak Dam, namely Strathgordon, which is 35 km distant, was obtained from the Bureau of Meteorology to provide an estimate of rainfall at Scott's Peak Dam.

2.3 Results

Table 2.1 shows the maxima, minima and appropriate dates for the environmental factors measured at the Scott's Peak Dam collection site, with some additional information on rainfall from Strathgordon.

Table 2.1 Range of some environmental factors at Scott's Peak Dam collection site

	Maximum	Date	Minimum	Date
Surface temperature (^{o}C)	39	Jan. '76	-3	June '76
Water/soil temperature (^{o}C)	25	Jan. '75	1	Sept. '76
Burrow temperature on collecting trips (^{o}C)	16	7/2/'78	3	28/5/'75
Oxygen concentration (mL/L)	7.9	9/8/'75	0.86	24/10/'75
pH	5.6	13/8/'76	3.7	24/10/'75
Mean monthly rainfall at Strathgordon* (mm)	314	May	103	Feb.

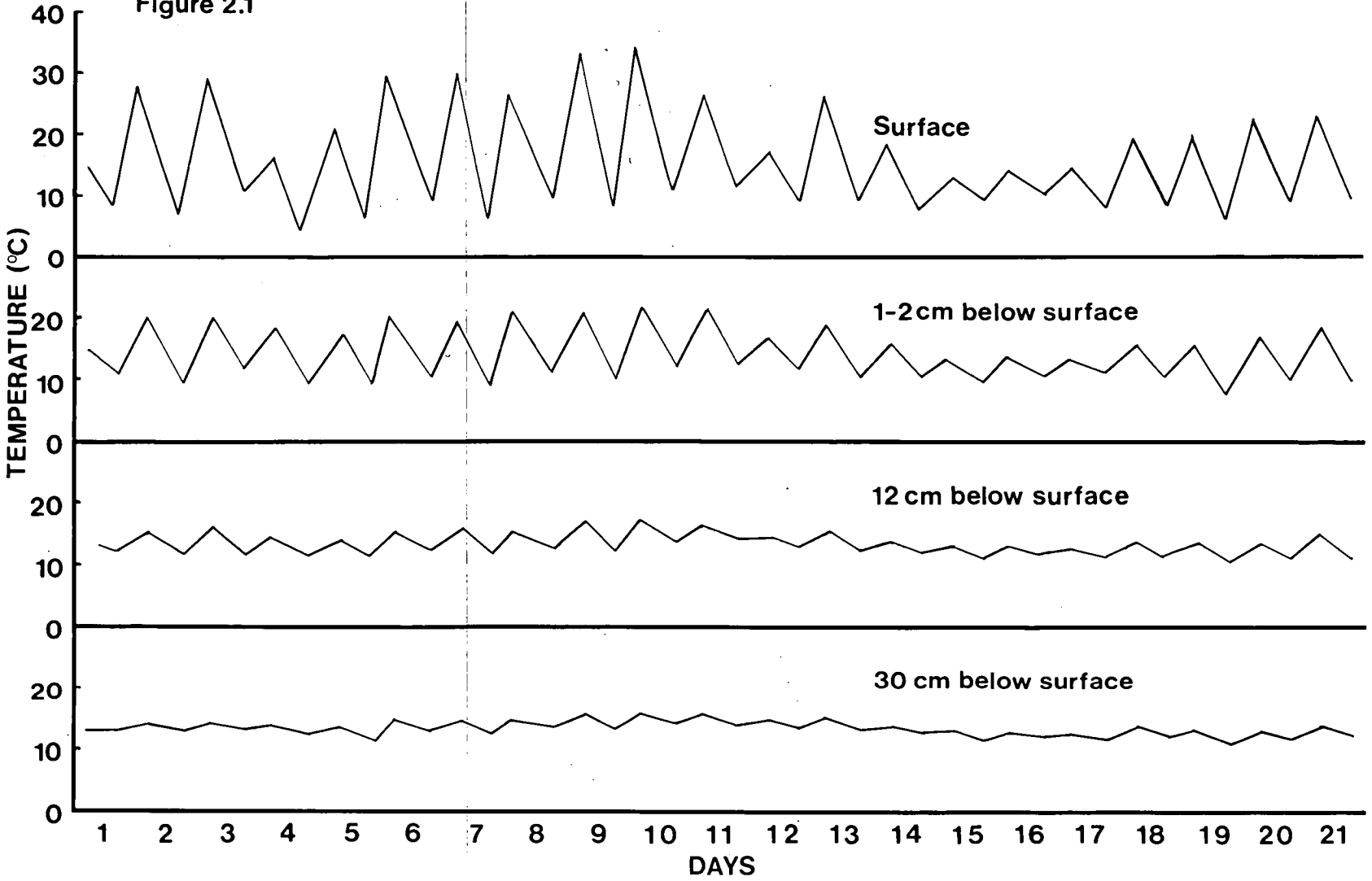
* 7 years' recordings

The maximum and minimum temperature recordings from the Grant recorder for the three week period starting on February 17, 1976 are shown in Figure 2.1.

On a number of trips it was found that there were a number of burrows, containing live animals, that had no water in them. These trips were February 17, 1976; February 13, 1977; January 9, February 7 and March 7, 1978. It is not suggested that the burrows were dry for the complete period from January 9 to March 7, 1978 but during summer and early autumn there are always periods during which there are severe shortages of water in crayfish burrows on the button grass plains. Rainfall data for Strathgordon showed that in the period from December 1975 to April 1978 there were 12 periods of 5 days or more in which no rain fell, and 18 periods of 7 days or more during which less than 5mm of rain fell. During these periods many crayfish burrows would dry out. It is probable that frequent, quite heavy rainfalls are required before all burrows have water in them. Some dry burrows were found on February 7, 1978 despite 20mm of rain only 2 days earlier.

Figure 2.1 Daily maximum and minimum temperatures on the surface and at three different depths, at the Scott's Peak Dam site, for a three week period commencing on 17/2/'76.

Figure 2.1


40
30
20
10
0
Surface
20
10
0
1-2cm below surface
20
10
0
12 cm below surface
20
10
0
30 cm below surface
TEMPERATURE (°C)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
DAYS

Over the three year period during which collections were made, 596 animals were collected from the study area. Of these, 427 were over 1 g weight, and 60.7% of these 'adults' were females. A weight distribution graph of the collected animals, cumulated over the three years and divided into two-monthly periods, is shown in Figure 2.2. A histogram of the weight distribution of the entire 427 animals over 1 g weight was also drawn (Figure 2.3). In species where animals in each year class grow at a fairly uniform rate it is sometimes possible to see age classes in length-frequency data, and as there is a very good correlation between carapace length and weight for *P. tasmanicus* (see Figure 2.5), any age classes present might be expected to show up in a weight-frequency distribution. However no identification of age classes is possible from Figure 2.3; nevertheless the data do provide a basis for useful comparison between male and female crayfish. It was considered permissible to cumulate the animals collected at different times of the year, as the weight and size of each animal does not change continually through the year. The yearly size increase and most of the weight increase occurs in one discrete 'jump', during the annual moult. Animals of less than 1 g weight moult more than once each year, and therefore these were not included in Figure 2.3, as their more 'continuous' size and weight increase could have blurred any age classes that were present.

Figure 2.4 shows the percentage of females over 3 g weight in berry or with attached young, during the course of a year. Only two females under 3 g weight were ever found in berry, so this weight was selected as the minimum weight of females to be included in the construction of the graph; it may also be regarded as a reasonable indication of the weight at which females became sexually mature. Table 2.2 gives the number and percentage of females over 3 g weight that were in berry, or which had attached young, on each of the collection dates. The mean percentage of females over 3 g weight that were in berry or carrying young in the period from May 30 to January 26 was 47.5% (56/118). This was the period during

Table 2.2 Seasonal change in numbers of berried females.

Year	MONTH												Collection Dates
	Jan	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
1975			5(0)*		11(2)			10(4)		11(6)		10(6)	3/3; 28/5; 9/8; 24/10; 1/12.
1976		12(0)	6(0)		13(6)			10(5)			13(6)		17/2; 30/3; 30/5; 13/8 23/11.
1977	12(5)		8(0)	9(2)		13(7)		15(3)		10(4)		11(4)	26/1; 15/3; 26/4; 8/6; 16/8; 18/10; 6/12.
1978	9(6)	10(3)	13(0)										9/1; 7/2; 7/3
TOTALS	21(11)	22(3)	32(0)	9(2)	24(8)	13(7)	-	35(12)		21(10)	13(6)	21(10)	211(69)

*Number of females over 3g weight (Number in berry or with attached young)

Figure 2.2 Weight-frequency distribution of male, female and juvenile *Parastacoides tasmanicus* collected between 26/3/'75 and 7/3/'78 (inclusive) at the Scott's Peak Dam site. The frequency of crayfish in each 1 g size class, for each two month period, is given as a percentage of the total number of animals collected during the relevant two month period.

Figure 2.2

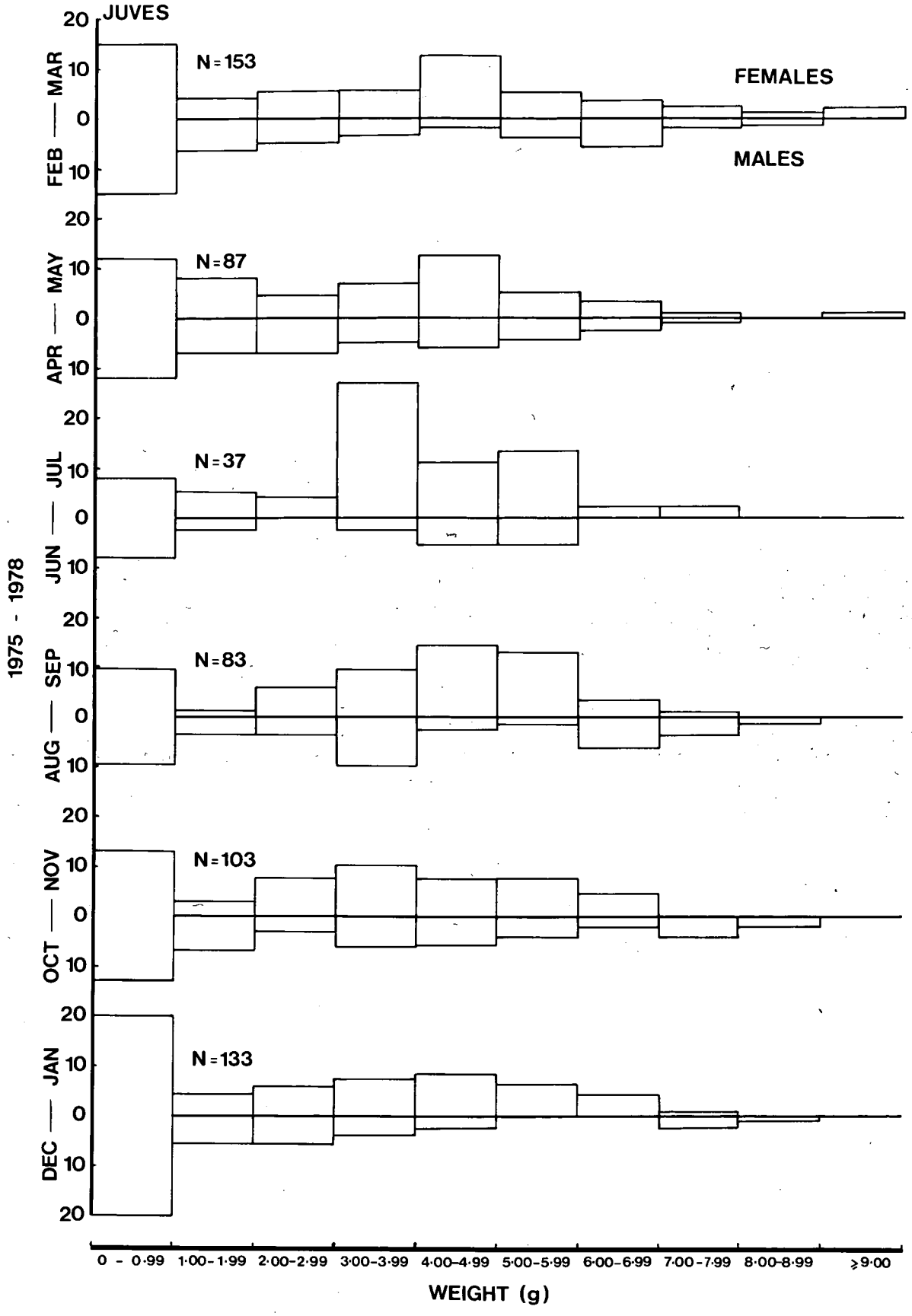

JUVES
FEMALES
MALES
N=153
N=87
N=37
N=83
N=103
N=133
FEB — MAR
APR — MAY
JUN — JUL
AUG — SEP
OCT — NOV
DEC — JAN
20
10
0
10
20
1975 - 1978
0 - 0·99
1·00-1·99
2·00-2·99
3·00-3·99
4·00-4·99
5·00-5·99
6·00-6·99
7·00-7·99
8·00-8·99
≥9·00
WEIGHT (g)

Figure 2.3 Weight-frequency distribution of male and female *Parastacoides tasmanicus* over 1 g weight collected at the Scott's Peak Dam site between 26/3/'75 and 7/3/'78 (inclusive). The crayfish collected over the entire three year period are grouped together.

FIGURE 2.3

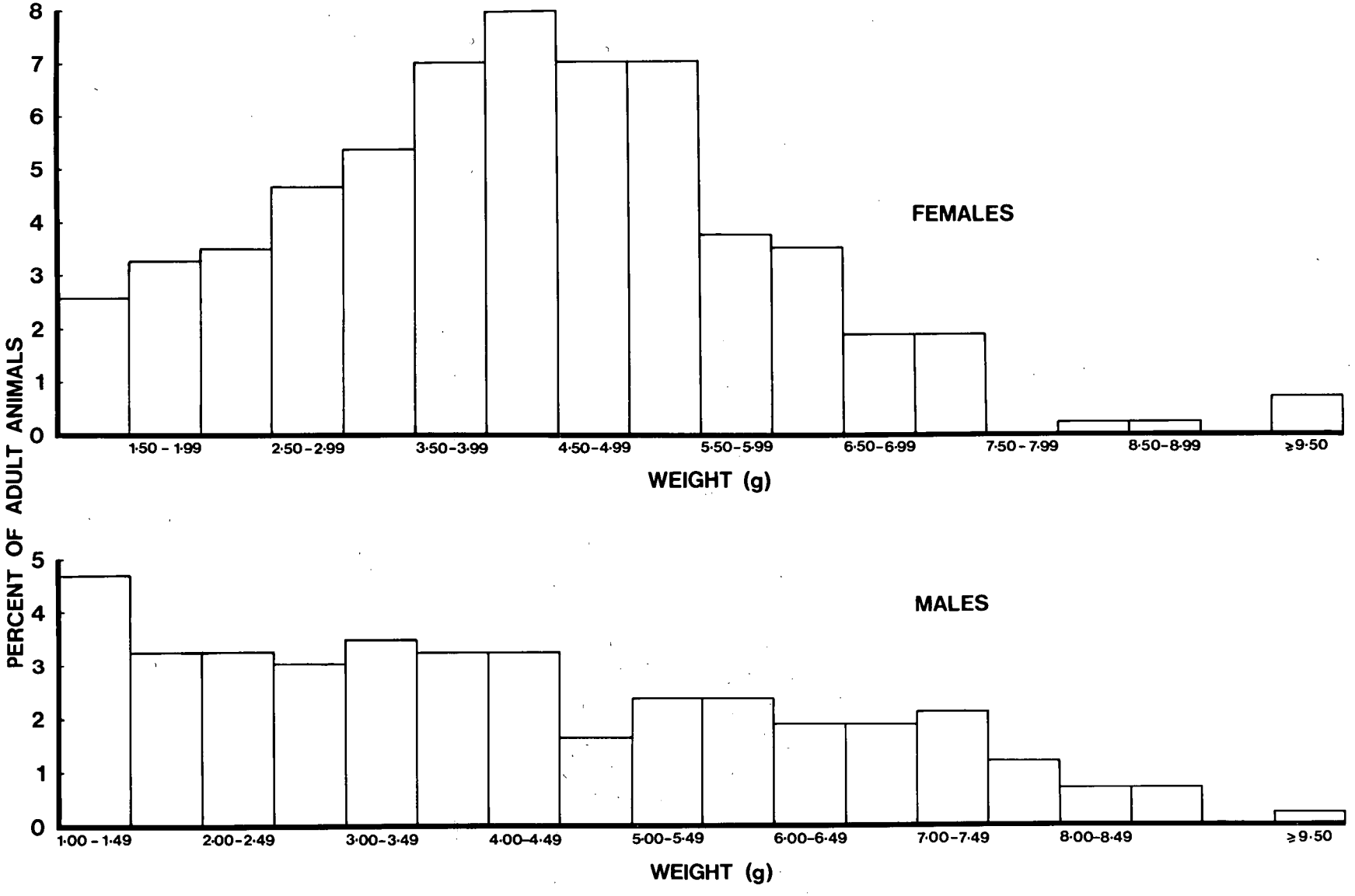

PERCENT OF ADULT ANIMALS
FEMALES
0
1
2
3
4
5
6
7
8
1·50 - 1·99
2·50 - 2·99
3·50 - 3·99
4·50 - 4·99
5·50 - 5·99
6·50 - 6·99
7·50 - 7·99
8·50 - 8·99
≥ 9·50
WEIGHT (g)
MALES
0
1
2
3
4
5
1·00 - 1·49
2·00 - 2·49
3·00 - 3·49
4·00 - 4·49
5·00 - 5·49
6·00 - 6·49
7·00 - 7·49
8·00 - 8·49
≥ 9·50
WEIGHT (g)

Figure 2.4 Seasonal variation in the percentage of female *Parastacoides tasmanicus* over 3 g weight in berry or with (at least one) attached young. Based on data presented in Table 2.2.

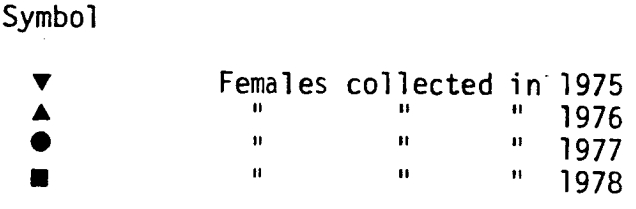
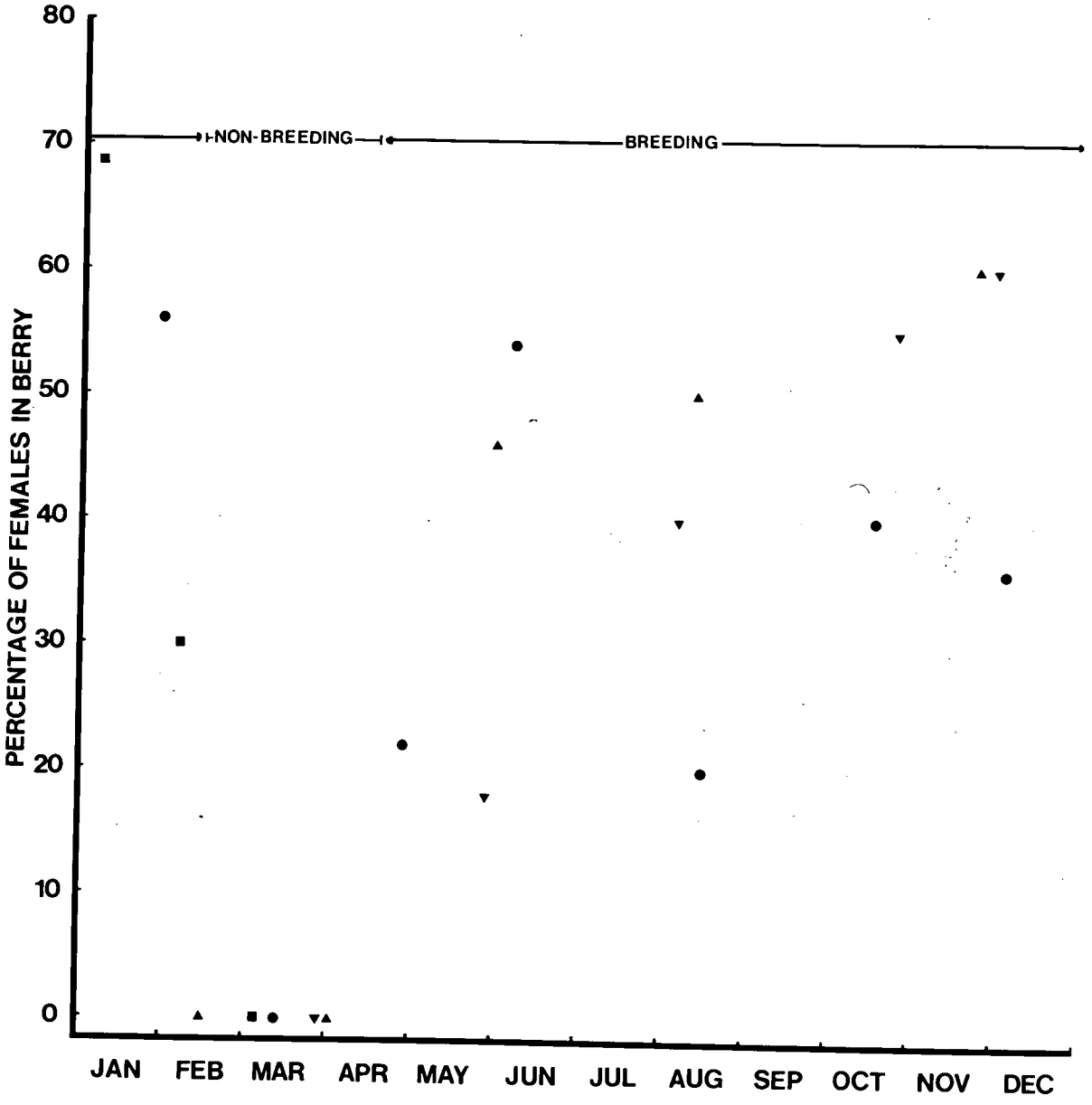


Figure 2.4


80
70
60
50
40
30
20
10
0
PERCENTAGE OF FEMALES IN BERRY
NON-BREEDING
BREEDING
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

which no more females came into berry and during which no berried female had lost all of her young, in the 3 years of the study.

Of the 24 burrows that were excavated and replaced minus occupants on April 26, 1977, 23 were found and excavated again on June 8. Nine of these were occupied. Ten of the 14 burrows dug up on August 16 were reoccupied by October 18. Table 2.3 shows the size (i.e. adults or juveniles) and sex of the animals in the recolonised burrows, along with other relevant data.

Table 2.3 Burrow recolonisation by *Parastacoides tasmanicus*.

	April 26 - June 8	Aug. 16 - Oct. 18
Number of burrows employed	23	14
Number (%) reoccupied	9 (39.1)	10* (71.4)
Time available for recolonisation (days)	43	63
Number (%) of juveniles	1 (11.1)	1 (8.3)
Number (%) of males	2 (22.2)	3 (25.0)
Number (%) of females	6†(66.7)	8 (66.7)

*Includes one burrow with three occupants

†Includes one berried female

The weights of the colonising animals ranged from 0.2 - 5.5 g for the animals collected on June 8, and from 0.5 - 5.0 g for the animals collected on October 18. There was no obvious relationship between the size of the animal and the size of the burrow that it had colonised, but it appeared that in general it was the larger burrows that had been recolonised.

On February 23, 1977, when the air temperature was 16^{o}C and relative humidity was 52%, the relative humidity 0·15 m into crayfish burrows was over 80%. When the great water holding capacity of the soil is considered (the soil can hold over 1.5 times its own weight of water - unreported data) it seems likely that even in burrows with no standing water, there would be sufficient moisture in the walls of the burrows to maintain the humidity in the deepest parts of the burrows at near 100%. It was not possible to measure the humidity in the deepest parts of the burrows, as the probe of

the humidity meter could not be moved very far into the convoluted tunnels of a burrow without contaminating the sensor.

The relationship between carapace length and weight is needed so that comparisons can be made between the results of this thesis, in which animals have been measured by weight, and other studies in which animals have been measured in terms of carapace length. The following reasoning results in a very good relationship being found.

Many animals have a shape that approximates that of a cuboid. If the three dimensions of the cuboid are linearly proportional to each other, then the volume of the cuboid will be linearly proportional to the cube of any of the dimensions. The shape of the crayfish approximates that of a cuboid, and its three dimensions are roughly linearly proportional to each other. For example, the relationship between the carapace length and the carapace width is expressed by the equation Carapace width = 0.506 Carapace length - 0.611 ($r = 0.995$, $N = 65$). Hence it might be expected that the carapace length, which is proportional to the total length, would be linearly related to the cube root of the volume of the animal. As the specific gravity of the animal is fairly constant over a wide size range, the carapace length might also be expected to be proportional to the cube root of the weight. This is in fact the case. An analysis of the linear regression of carapace length (in millimetres) on the cube root of the weight (in grams) of *P. tasmanicus* juveniles, males and females (excluding berried females) gave the following results. Carapace length = $0.379 + 13.456\ \text{Weight}^{1/3}$ ($N = 69$, $r = 0.993$, $F = 4804$). Normally a relationship between carapace length and weight would be given in the form Weight = $a + b\ \text{Carapace length}^{c}$, but in the form given above it is easier to make conversions of weight given in this thesis to carapace lengths.

A comparison of the goodness of fit of this model with 8 other models given in a family regression package on a Hewlett Packard 9825A calculator showed that this model was better than any of the other models. The best of these other models were

Carapace length = 6.494 + 7.373 Weight$^{\frac{1}{3}}$ (N = 69, r = 0.988, F = 2700)

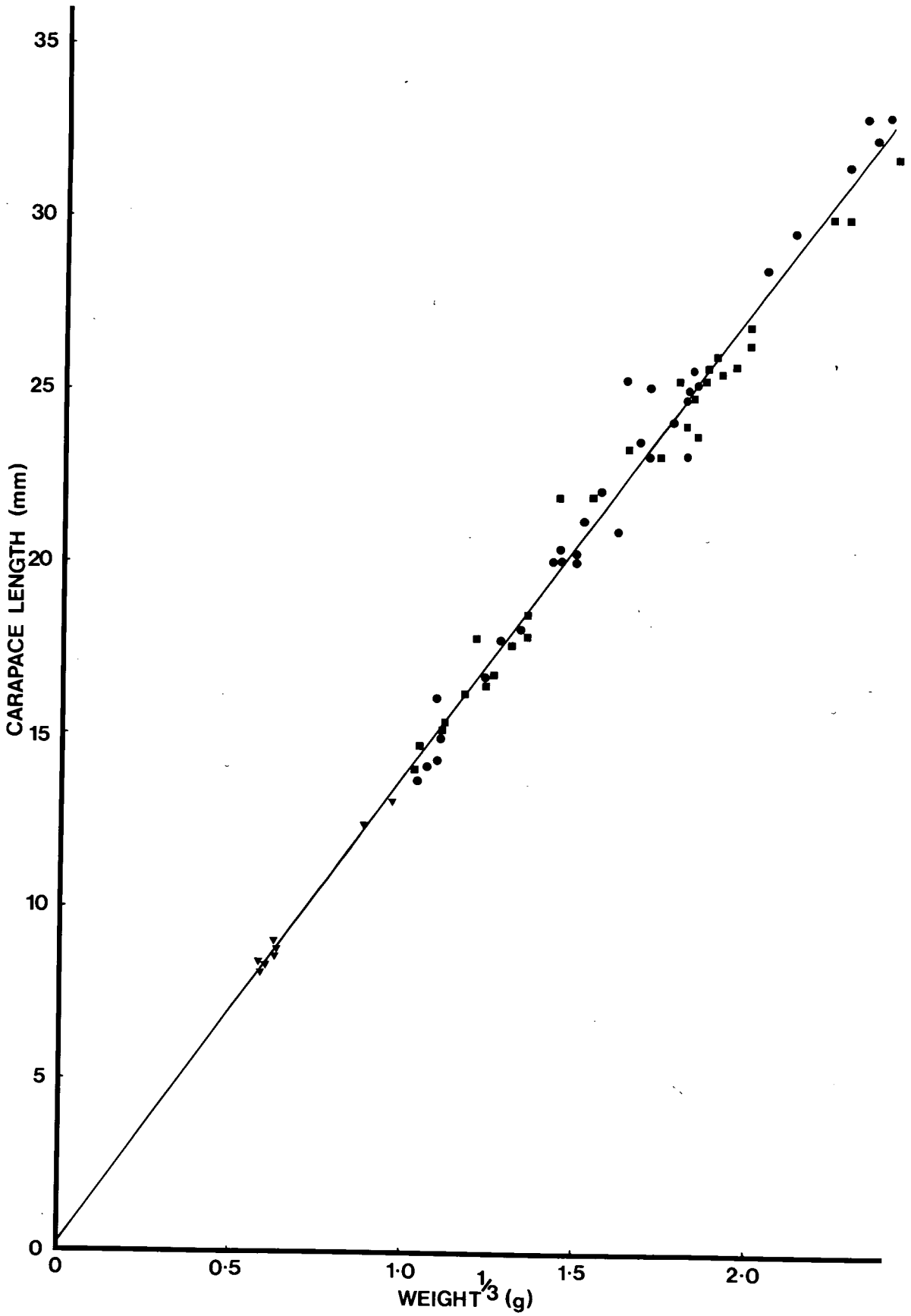
and

Carapace length = 14.599 + 5.774 ln Weight (N = 69, r = 0.972, F = 1144).

Neither of these two relationships is as good as the relationship between carapace length and the cube root of the weight. Figure 2.5 is a graph of carapace length against the cube root of the weight for male, female and juvenile *P. tasmanicus*. There is little difference between the relationship for male and female *P. tasmanicus*.

Figure 2.5 Relationship between carapace length (mm) and weight$^{1/3}$ (g) of female (●), male (■) and juvenile (▼) *Parastacoides tasmanicus* collected at the Scott's Peak Dam site.

Figure 2.5



2.4 Discussion

Table 2.1 shows that surface temperatures on the button grass plains may reach very high values during summer (39°) and yet may drop below freezing during winter (-3°C). At a depth of 0·10 -0·15 m the temperature range during the year is not quite so large, but summer temperatures still reach 26°C and winter temperatures drop to as low as 1°C. The recordings from the Grant temperature recorder showed that at a greater depth the daily (and seasonal) changes were even less pronounced. On one day, when the surface temperature ranged from a low of 8.5°C to a high of 34°C the temperature at a depth of 30 cm only ranged from 13° to 15.5°C. Over a three week period the surface temperature ranged from 4.5° to 34°C but the temperature range at 30 cm depth was only 11° to 15.5°C. On collecting trips burrow temperatures at a depth of about 40 cm only ranged from 3° to 16°C, although a maximum range of a few degrees above this, to a degree below it could be expected. Lake and Newcombe (1975) measured burrow temperatures at a depth of 10 cm of 5° to 15°C between mid-February and mid-November, at their site in the South-West.

In brief, crayfish would never be troubled by sub-zero temperatures at depths of more than a few centimetres, although they could have to face a combination of lack of standing water and relatively high temperatures during summer if their burrows were not very deep. Crayfish with burrows over 30 cm deep would never have to face extremes of temperature unless they ventured into surface parts of their burrows during very hot or very cold times.

The pH range that was measured during collecting trips (3.7-5.6) is probably an underestimate, although it is a wider range than the 4.4 ± 0.3 found by Lake and Newcombe (1975). During prolonged

wet periods, when the humic acids in the soil are diluted, the pH might be expected to rise, and could reach values approaching pH 6 During dry, hot periods, there would be very little water flow through the button grass plains, and yet humic acid production could probably be at maximum levels. The pH may drop below the lowest measured value of pH 3.7, but it is at these times that it is very difficult to obtain water samples with which to measure the pH.

During dry, hot periods, not only might one expect the pH of burrow water to decrease, but one might also expect the oxygen level of the water to decrease, due to decreased solubility, and an increase in the rate of plant decomposition and respiration rate of plants and animals. The lowest oxygen level that was recorded in burrow water was 0.86 mL/L, similar to the lowest level found by Lake and Newcombe (1975) of 1 mg/L (0.7 mL/L), but it is quite reasonable to assume that levels below this often occur. Low levels of oxygen would coincide with the times when oxygen measurements could not be made due to a lack of sufficient clean water, or when the humic acid levels in the water were high enough to affect oxygen measurements by the micro-Winkler technique. It is reasonable to assume that completely anoxic conditions could occasionally occur in burrow water, and severely reduced oxygen levels could often occur in summer, and to a lesser extent in winter, between rains when the water is stagnant.

Rainfall at Scott's Peak Dam is probably similar to that at Strathgordon. The area receives over 2500 mm rain/year, with dry periods occurring occasionally during summer, and prolonged wet periods occurring during the rest of the year, especially in winter.

The size distribution graphs of the crayfish population at different times of the year (Figure 2.2) demonstrate that there is

little recruitment of juveniles into the population. From a peak in juvenile numbers in December-January, when the majority of juveniles are leaving the females, the percentage of juveniles in the population decreases steadily until October-November (June-July measurements have been disregarded due to the small sample size), when the following year's juveniles start to leave their mothers. However, the next smallest size class shows no appreciable increase at any stage of the year, the only suggestion of an increase in this class being in April-May and even that apparent increase could be an artifact caused by the small sample size.

There could be some bias in the size distributions of the animals, as juveniles, especially the very small ones that have just left their mothers, are very difficult to find in burrows with muddy water. Consequently the December-January and the February-March juvenile numbers are probably larger than the collections suggest. However, the numbers of juveniles collected later in the year could be closer to the correct values, as the juveniles grow during the summer and autumn (although they are still less than 1 g weight) and are easier to find.

Figure 2.3 shows that there are no distinct size classes in the size distribution of either male or female *P. tasmanicus* which might suggest age groups, probably because animals in any year class grow at different rates. Newcombe (1970) suggested that his sample of 157 animals showed a possible 8 size classes, but it is more likely that his distribution was due to chance rather than to the presence of genuine size classes. This is especially likely in view of the fact that he grouped males and females together, yet as will be shown later (Chapter 6), and as Figure 2.3 suggests, the growth rate of males is probably faster than that of females, so that combining data from both sexes would tend to mask any possible size classes.

The variable growth rates observed in *P. tasmanicus* also occur in *Cherax destructor* (B. Mills, pers. comm.) so *P. tasmanicus* is certainly not unique among the parastacids in this respect. Figure 2.3 shows that most of the adult female crayfish collected were in the 3 to 6 g weight range. Growth to about 3 g might be expected to be fairly rapid, but would then slow down, as the mature females would divert a lot of energy into egg production once they exceeded this weight. This would result in the large percentage of slow growing females in the 3 to 6 g weight range, with only a small proportion of the females living long enough, or growing fast enough to reach a weight above 6 g. It is possible that the few females that exceeded the weight of 8 g had not been breeding for some reason.

The male crayfish did not show any accumulation of numbers in any size range, but showed a slow decrease in numbers of animals with increasing size, with some animals dying in each size group (each year). It appears that most of the animals that live to reach the size of 1.5 g then survive to the size of 4.5 g, after which mortality increases to some degree, with very few animals reaching a size of greater than 8 g. It is perhaps not surprising that on an average males grow to a larger size than females, as they do not have to produce eggs and as will be shown in Chapter 6, females only have a moult in which they increase in size once every two years compared to the males' once a year. The percentage of males in the over 6 g size group is 51%, as compared to 37% in the under 6 g size class, with an overall average of 39.3%.

Lake and Newcombe (1975) estimated that the percentage of berried females among mature females in August-September was about 40%. This agrees with estimates in this thesis of 47.5% for the period between May 30 and January 26. In this period in 1975/'76, 1976/77 and 1977/'78, 51.6% (16/31), 45.8% (22/48) and 41.4% (24/58) respectively of the sexually mature females were in berry. However, Lake and Newcombe found that animals in their study site became mature at a carapace length of at least 24mm, which is equivalent to a weight of over 5 g (from Figure 2.5), whereas the females at the Scott's

Peak Dam site became sexually mature at about 3 g which corresponds to a carapace length of 19.8mm. They also found that juveniles left their mothers in February-March, whereas at Scott's Peak Dam the juveniles had all left their mothers by mid-February. These differences may be due to slight differences in climatic conditions in the two areas and/or differences in the populations at the two sites.

There is also disagreement on one other point. Lake and Newcombe suggested that there was a low level of fertilization amongst *P. tasmanicus*, which was why only 40% of the females were in berry each year. The real reason is that *P. tasmanicus* females are only capable of breeding once every two years (see Chapter 6), so the maximum percentage of females that could be in berry at any time is only 50%.

From the rapid rate at which emptied burrows were reinhabited, it appears that *P. tasmanicus* must spend a considerable amount of time above ground, moving about (at least in the periods tested). This movement probably occurs at night. Furthermore, this rapid reoccupation of burrows occurred during late autumn, and again in early spring, when animals are considered to be less active than they are in warmer months. What is more surprising is that adults move around as well as juveniles, yet only juveniles would be expected to have to search for empty burrows. Although single measurements of reoccupation rates in autumn and in spring do not permit speculation about seasonal variation in reoccupation rates, some comment on sex ratios is permissable. The male to female ratio is close to what would be expected from the normal ratio of the two sexes, but there does appear to be a shortage of berried females. This is not unexpected, as berried females are usually considered to be less active than other animals.

From the rate at which the empty burrows were recolonised, it might be expected that many animals wander around on the surface on suitable nights, occasionally entering burrows. If an animal entered an inhabited burrow, one of the two crayfish would be driven out, usually without damage as described by Leggett (1971), and would have to search for an unoccupied burrow. Once it found an unoccupied burrow, the burrow might become its new (temporary) home. Animals that spent winter in an area that became uninhabitable in summer would have to search for a new burrow or run the risk of perishing in the old one. Newcombe (1970) measured the relative humidity on the button grass plains during the day and at night, in April, and found that even though the daytime humidity might have been only 50%, the humidity at night was 95%, high enough to permit crayfish to spend some time out of their burrows without any danger of desiccation.

The purpose of Figure 2.5 is merely to permit comparisons to be made between animals collected at Scott's Peak Dam, which were measured by weight, and *P. tasmanicus* or other crayfish that were measured by carapace length as was the case with Newcombe (1970) or Lake and Newcombe (1975).

Very little has been said here about comparisons of *P. tasmanicus* with other freshwater crayfish and other animals. Much more will be said in the appropriate chapters. As stated previously, this chapter was merely intended to be a brief outline of some aspects of the ecology of *P. tasmanicus*.

3. SOME EFFECTS OF pH ON *Parastacoides tasmanicus*

3.1 Introduction

Newcombe (1975) showed that *P. tasmanicus* can survive indefinitely in water with a pH of 2.75, and for more than 130 hours at pH 13.0. However, it is not known whether survival at the extremes of this pH range places the animal under considerable stress, or whether the animal is relatively indifferent to the pH.

Ivanova (1969) wrote "The studies concerning the reaction of aquatic animals to changes in a given environmental factor and the determination of the optimal values of this factor can be achieved....by measurements of the feeding rate or respiration rate in different conditions." Feeding rate may be a suitable variable to measure in some continuous feeders, and has been used successfully with cladocerans (Kring and O'Brien, 1976), but measurement of respiration is usually a more direct and applicable method of determining optimal conditions, especially in sporadic feeders.

Measurements of routine oxygen consumption rates ($\dot{V}O_2$) of prosobranch snails (Buckingham and Freed, 1976) and rainbow trout (Hargis, 1976) and of the respiratory rhythm of a freshwater crab (Tyagi, 1973) over a wide range of pH, all show the same sort of pattern. If the pH is altered from the optimal pH range of the animal concerned, its $\dot{V}O_2$ (or rate of water expulsion from the branchial chambers) increases almost immediately, and stays elevated (for a time at least). The $\dot{V}O_2$ increases further as the pH is raised or lowered further. This increased $\dot{V}O_2$ shows that it is energetically stressful, although not necessarily harmful, for the animal at these pH*s*. If the pH of the water in which the animal is contained is raised or lowered still further, the tolerance limits of the animal are eventually

exceeded, and at higher or lower pH the VO_2 is depressed. It could be suggested that this is due to the failure of the oxygen transport system, or due to damage to the gills or some other system. As the pH is increased or decreased even further, damage is more extensive and more rapid, and the VO_2 is further depressed.

Experiments on other animals have given different results from those just mentioned. Ultsch (1978) found that the respiration of three species of fish was unaffected at pH 7.0 and pH 4.0 although pH 4.0 was lethal to two of the species tested. Even at pH 3.5 the oxygen consumption of only one of the fish was reduced before death occurred.

Despite these differences, a graph of VO_2 against pH may be expected to provide some insight into how an animal copes with a range of pH. If the graph shows an almost unvarying VO_2 over a wide pH range, it suggests that the animal is not energetically stressed over the pH range concerned. However, it does not show that the animal is necessarily tolerant of the pH range concerned. If there is an optimal pH range, then an increase in VO_2 outside of this optimal range shows how much the animal is capable of increasing its oxygen consumption in response to external pH, possibly in an attempt to reduce or prevent the damage caused by the hydronium or hydroxyl ions.

The effects of hydrogen ion concentration on living organisms at a molecular level are reasonably well known. The conformation and activity of enzymes and other macromolecules can be drastically altered by small changes in pH (Albert, 1952; Bittar, 1964; Giese, 1968; Malan, Wilson and Reeves, 1976; Masoro and Siegel, 1971) and it is to prevent these changes that the pH of the circulatory fluids of most animals is kept within a restricted range. For example, the pH of the haemolymph of gastropods is in the range 7.5 - 8.2 (Burton, 1969) while the pH of invertebrate body fluids in general is between 6.7 and 8.1 (Giese, 1968; Howell *et al*, 1973; Mangum

and Shick, 1972; Miller, Pritchard and Rutledge, 1976; Punzo, 1977). The pH of body fluids of both invertebrates and ectothermic vertebrates changes slightly with changes in temperature and other external factors (Eddy, 1974; Howell *et al,* 1973; Howell and Gilbert, 1976; Mangum *et al,* 1976; Randall and Cameron, 1973; Truchot, 1973a, 1973b). For example, the pH of the prebranchial haemolymph of the crab, *Carcinus maenas,* in air is 7.94 ± 0.05 at 6°C, but it is 7.64 ± 0.03 at 22°C (Truchot, 1973a). Intracellular pH is also maintained within limits in ectotherms, although temperature and other factors do affect it to some extent (Malan *et al,* 1976; Reeves and Malan, 1976).

It is possible for external pH to produce large changes in body fluid pH, with adverse effects on the organism concerned. For example, the cause of death of some fish exposed to low pH is acidaemia (Lloyd and Jordan, 1964). Another cause of death in fish exposed to low pH is the loss of sodium, but not potassium from the body (Dunson, Swarts and Silvestri, 1977; Packer and Dunson, 1970, 1972).

The causes of death of invertebrates exposed to low pH has not been conclusively determined, although there are some indications that the causes may be similar to those that occur in fish. For example, Shaw (1960) found that sodium influx in *Austropotamobius pallipes* was inhibited at pH below 6.0, although efflux of sodium was not affected. This resulted in a net loss of sodium at low pH. It is not known if acidaemia occurs in invertebrates exposed to low pH.

As stated earlier, *P. tasmanicus* lives in an acid environment, and it should be profitable to study the effects of a wide range of pH on it. Measurements of the effect of external pH on the haemolymph pH and survival of *P. tasmanicus* should provide an indication of the effect that external pH has on internal pH, and

the range of internal pH that can be tolerated by this crayfish. A measurement of the loss of sodium and potassium by *P. tasmanicus* in normal and very low pH should indicate whether death in this species at very low pH is associated with the loss of considerable amounts of either of these ions, while measurements of the effect of external pH on oxygen consumption of *P. tasmanicus* should show whether or not pH affects the VO_2 of the crayfish in the same way that it affects the VO_2 of any of the animals mentioned earlier.

3.2 Materials and methods

Adult *Parastacoides tasmanicus* that were in the C stage of the moult cycle, as determined by the method of Mills and Lake (1975), were caught and maintained as described in Chapter 2. Animals were kept at both 5° and 15°C, in button grass water with a pH of 4.5 - 5.5.

3.2.1 The effect of external pH on the oxygen consumption of *Parastacoides tasmanicus*

A 'closed bottle' technique was used to measure the VO_2 of the animals that had been maintained at 15°C. Each animal was placed in a glass jar that had a wide flat lip. The volume of the jars used depended on the size of the animals, but all had a capacity of between 500 and 1400 mL. The jars were filled with water (see below) of a known pH and left for several hours to allow the animals to settle down and overcome any initial 'shock'. A water sample of 2.0 mL was taken from each jar and the initial oxygen concentration determined by the micro-Winkler method (Fox and Wingfield, 1938). The jars were then closed with perspex lids which were held in place with vacuum grease, with care being taken to ensure that no air bubbles were trapped under the lids. The jars were placed in the dark at 15°C and left for 24 hours, after which time the water in them was thoroughly mixed by inverting the jars several times. The jars were opened and several water samples were taken for oxygen determinations. The pH was also checked.

The oxygen concentration of the water in the jars never dropped below 4.5 mL O_2/L, and consequently would not have exerted any effect on the oxygen consumption of the crayfish (see Chapter 7).

Before experiments on animals kept and tested at 5°C were commenced, a Gilson Differential Respirometer became available, and this apparatus was used for all VO_2 measurements at 5°C. The

technique used was that described in the Gilson manual, using specially designed 300 mL. flasks two-thirds filled with water. Trial studies indicated that a three hour equilibration period was satisfactory, after which the oxygen consumption of animals was measured for a further three hour period, between 12 noon and 3 p.m. The apparatus allowed the VO_2 of five animals to be measured simultaneously.

The water used in all of these experiments was water from Lake Pedder which had been treated with 25 mg/L of both streptomycin and neomycin, and then filtered. This concentration of antibiotics was found to adequately inhibit bacterial oxygen consumption without being harmful to the crayfish, and had the added advantage that it precipitated most of the organic matter in the water, which could be filtered off, leaving an almost colourless liquid. The large amount of precipitate and the dramatic drop in absorption of the water at 440nm (one of the wavelengths which the colouring matter in the water absorbs (see Kirk, 1976)) showed that most of the colouring matter (including the organic matter) had been removed. The pH of the water was adjusted to the required pH with either 0.1 M hydrochloric acid or 0.1 M sodium hydroxide (1 M sodium hydroxide was necessary for pH above 11). The pH was measured with a Townson Digital pH meter. The water was aerated for at least two hours, and the pH rechecked, before it was used.

Due to the limited number of animals available for these experiments, and also to reduce variation resulting from the use of different animals in each test, each group of animals was utilized for a series of pH. The VO_2 of animals at $15^{o}C$ was measured over a range of pH from 1.9 to 13.2. pH 1.9 is below the lower lethal limit of *P. tasmanicus* and pH 13.2 is above the upper lethal limit of the crayfish, according to Newcombe (1975). The animals at $5^{o}C$ were tested at a number of pH between 2.6 and 11.3. pH 2.6 is about the lower lethal limit of *P. tasmanicus* and pH 11.3 is slightly below the upper lethal limit. The animals were tested at random at each pH, and at least one week was allowed between tests, in order

to allow the animals to recover from any possible ill effects of a previous test, although no indication of any such effects e.g. abnormal locomotory or feeding behaviour or appearance, was ever observed. None of the animals died during, or within one month of, the tests.

3.2.2 The effect of external pH on haemolymph pH of *Parastacoides tasmanicus*

The effect of pH on haemolymph pH was tested with a pH series of 2.20, 3.00, 3.25, 5.00, 6.70, 8.80, 11.00 and 12.60; three animals were kept at each pH and all of the tests were carried out at 15^{o}C. The range chosen includes a pH above the upper lethal limit and one below the lower lethal limit. After 110 hours in the test water the animals were removed, washed in tap water, and samples of 0.1 mL of haemolymph taken from the base of the 5th pair of pereiopods with 1 mL syringes fitted with 23 gauge needles. The haemolymph samples were expelled onto pH 6.4 - 8.0 narrow range indicator paper, and the pH was determined as accurately as was possible. It is recognised that this is not an ideal method of pH measurement, but unfortunately no more sensitive procedure was available for the small volumes of haemolymph obtainable. Comparisons between this technique and standard electrode measurements of known solutions indicated that with experience an accuracy of ±0.1 pH unit could be achieved. This was more than adequate for present purposes.

3.2.3 The effect of external pH on loss of sodium and potassium ions from *Parastacoides tasmanicus*

To determine whether or not *P. tasmanicus* loses sodium and/or potassium at very low pH, 8 animals were each placed in 205 mL of water, in individual 1 L plastic containers at 15^{o}C. Four of the crayfish were exposed to a pH of 2.5, and the other 4 to a pH of 4.8. pH 2.5 is below the lower lethal limit of *P. tasmanicus* while pH 4.8 is in the normal pH range. A 5 mL water sample was taken from each container at the start and finish of a 72 hour exposure period. The samples were diluted appropriately with distilled water, and their sodium and potassium concentrations measured using an Eel flame photometer.

3.3 Results

3.3.1 The effect of external pH on oxygen consumption of *Parastacoides tasmanicus*

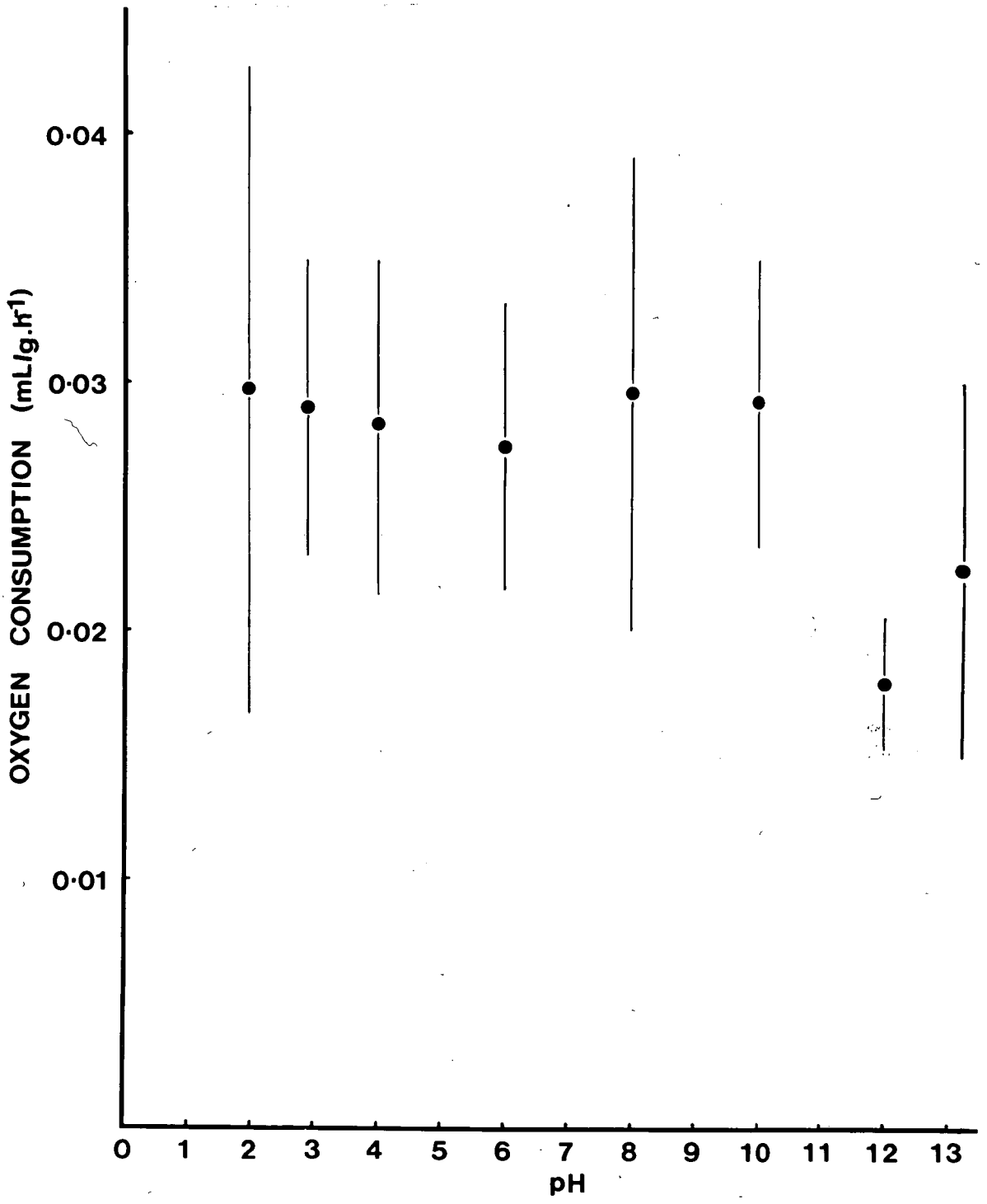
The effect of pH on the VO_2 of 6 *P. tasmanicus* acclimated and tested at 15°C is shown in Figure 3.1. Unfortunately the relationship between oxygen consumption and weight of the animals tested was not good enough to allow the oxygen consumption at each pH to be expressed in the form VO_2 (mL $O_2/g.h^{-1}$)=aWb^{-1} where w is the weight in grams and a and b are empirically determined constants. Therefore thegraph of oxygen consumption against pH gives the oxygen consumption as mean ± S.E. The crayfish used in this experiment had a size range of 2.39 - 11.91 g (mean ± S.E. was 5.75 ± 1.49 g).

At 15°C there were no statistically significant differences between the VO_2 of animals over a range of pH from 1.9 to 10.0. However, at pH 1.9 none of the VO_2 measurements were near the mean value of 0.0297 mL $O_2/g.h^{-1}$. They were all either very high (greater than 0.038 mL $O_2/g.h^{-1}$)or very low (less than 0.0220 mL $O_2/g.h^{-1}$) and thisis reflected in the very large standard error obtained. The elevated values at this low pH presumably result from the greatly increased locomotor activity demonstrated by the crayfish concerned. Other crayfish started to show symptoms of distress very quickly, and these animals account for the low oxygen consumption measurements obtained. Exposure beyond the experimental period of 24 hours to pH less than about 2.75 (the lower lethal pH) would probably result in reduced oxygen consumption in all specimens, as they succumbed to the low pH.

Above pH 10.0 the oxygen consumption is depressed and there is a significant difference between the VO_2 at pH 12.0 and the mean of the VO_2 between pH 3.0 and 10.0 (t-test : $P < 0.05$). The increased VO_2 at pH 13.2 is not statistically different from the VO_2 at pH 12.0, and at higher pH the VO_2 might be expected to decrease, as the respiratory structures of the crayfish would be rapidly damaged by the alkaline conditions.

Figure 3.1 Relationship between oxygen consumption of *Parastacoides tasmanicus* and external pH, at 15°C (Mean ± S.E., N = 6).

Figure 3.I


0·04
0·03
0·02
0·01
OXYGEN CONSUMPTION (mL/g.hr⁻¹)
0 1 2 3 4 5 6 7 8 9 10 11 12 13
pH

The effect of pH on the VO_2 of crayfish at 5°C is shown in Figure 3.2. As only five crayfish could have their oxygen consumption tested at any one time, each point on the graph represents the mean ± S.E. of the oxygen consumption of 5 crayfish. The weight of the crayfish used ranged from 2.07 to 10.99 g (mean ± S.E. was 4.85 ± 0.71g). As at 15°C, the VO_2 is not affected over a wide range, in this case pH 2.7 to 7.6. Above pH 7.6 the mean oxygen consumption decreases as the conditions become more alkaline, and at pH 9.5 the VO_2 is significantly different from the mean of the VO_2 values between pH 2.7 and 7.6 (t-test : $P < 0.05$). The rapid rise in VO_2 at elevated pH (pH greater than 9.5) is not statistically significant.

At both of the temperatures tested the VO_2 was not affected by pH in the range of pH 2.7 - 7.6, which is a much wider range than *P. tasmanicus* would normally encounter.

3.3.2 The effect of external pH on haemolymph pH of *Parastacoides tasmanicus*

The relationship between external pH and haemolymph pH at 15°C is shown in Table 3.1. Five animals, 2 at pH 2.20 and 3 at pH 12.60, died during the test period. These observations agree with the results of Newcombe (1975), who found that crayfish at pH 2.00 only survived for about 100 hours, and that one animal exposed to pH 2.25 survived for 177 hours. He also found that 3 animals at pH 13.0 survived for about 130 hours, which is somewhat longer than the animals at pH 12.60 survived in the present test.

Table 3.1 shows that although *P. tasmanicus* can tolerate a very large external pH range at 15°C, its internal pH remains fairly stable. It does not appear that this animal has been required to develop a tolerance to a wide range of haemolymph pH as part of its adaptation to a wide range of external pH.

Figure 3.2 Relationship between oxygen consumption of *Parastacoides tasmanicus* and external pH, at 5°C (Mean ± S.E., N = 5 for all measurements).

Figure 3.2

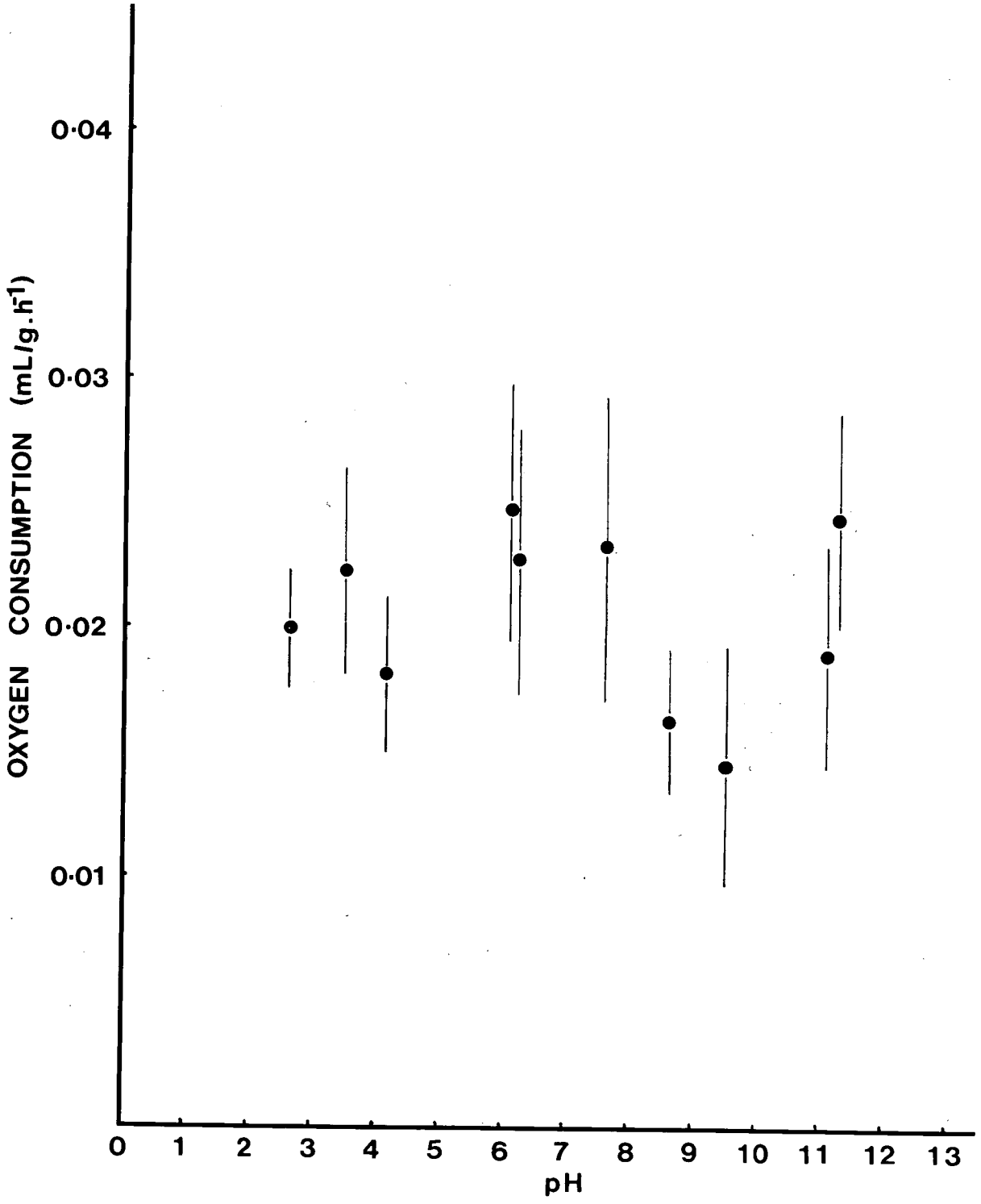

OXYGEN CONSUMPTION (mL/g.h-1)
0·04
0·03
0·02
0·01
0
1
2
3
4
5
6
7
8
9
10
11
12
13
pH

Table 3.1 Effect of external pH on the haemolymph pH of *Parastacoides tasmanicus*

External pH	Haemolymph pH after 110 hours		
2.20	6.8	6.7-	-
3.00	6.8	7.0	7.0
3.25	7.0	7.0	7.0
5.00	7.0	7.0	7.1
6.70	7.1	7.1	7.2
8.80	7.0	7.2	7.2
11.00	7.2	7.2	7.2
12.60	7.5-	-	-

- denotes a dead animal at the end of the exposure period

3.3.3 The effect of external pH on loss of sodium and potassium ions from *Parastacoides tasmanicus*

The amount of potassium and sodium lost by *P. tasmanicus* in moderately and strongly acidic media during the 72 hour test period is shown in Table 3.2.

Table 3.2 Sodium and potassium lost by *Parastacoides tasmanicus* in moderately and strongly acidic media during a 72 hour exposure period.

External pH	2.5	4.8	t-test
Animal wet weight (g)*	5.49 ± 0.99	6.00 ± 0.32	No sig.diff.
Potassium loss* (μmol/g wet weight)	0.82 ± 0.08	0.82 ± 0.41	No sig.diff.
Sodium loss* (μmol/g wet weight)	18.96 ± 5.05	5.70 ± 1.70	P<0.05

* All values as mean ± S.E. of 4 animals

Parastacoides tasmanicus specimens, in water with a pH of 2.5, lost a considerable amount of sodium, although they lost no more potassium than did the control animals at pH 4.8. In fact, if the blood sodium level of normal animals is assumed to be approximately 244 mmol/L (see Chapter 7), and if it is assumed that the haemolymph volume of *P. tasmanicus* is approximately 20% of the body weight, then a loss of 18.96 μmol sodium/g wet weight is equivalent to a loss

of almost 39% of the total sodium in the haemolymph. The slight loss of sodium by the 'control' animals ($p < 0.05$) (i.e. the animals at pH 4.8) and loss of potassium by the test animals ($p < 0.05$) could be due to the fact that the animals were not acclimated to the test solutions before the experiment commenced, but there is no possibility that the loss of sodium by the test animals was an artifact. Even if 5.70 µmol sodium/g wet weight is deducted from the sodium loss by the animals in water of pH 2.5, i.e. the amount of sodium 'lost' by the controls, the remaining sodium loss is still equivalent to over 27% of the total haemolymph sodium.

Thus it is apparent that *P. tasmanicus* loses sodium from its body at lethally low pH (i.e. pH of 2.5 or possibly even 2.75, and below), and this is very likely to be a major factor contributing to the death of this crayfish at an acidity outside its tolerance limit.

3.4 Discussion

The relationship between oxygen consumption and pH in *P. tasmanicus* differs markedly from that of the prosobranch snail, *Viviparus contectoides* (Buckingham and Freed, 1976), and the other animals mentioned in Section 3.1. *Parastacoides tasmanicus* exhibits little variation in metabolism over a wide range of pH (2.75 - 10.0 at 15°C and 2.6 - 7.6 at 5°C) and the pH of its haemolymph also shows little variation within this range. At higher pH values the VO_2 decreases, and the animal does not increase its VO_2 significantly (although larger samples may show this) even at very high pH i.e. pH 13.2 at 15°C and pH greater than 9.5 at 5°C. At very low pH there is no increase in VO_2, except that due to an increase in activity. The haemolymph pH is not greatly affected at any of the pH tested, although further tests need to be done at pH 2.5 and less, and at pH 12.0 and above.

By way of contrast, the snail, *Viviparus contectoides* (Buckingham and Freed, 1976), the rainbow trout, *Salmo gairdneri* (Hargis, 1976), and the crab, *Paratelphusa masoniana* (Tyagi, 1973) all have narrow 'optimal' pH ranges for the external medium of approximately 2 pH units. If the pH alters slightly from these ranges the VO_2 increases almost immediately. However, the respiration of the fish tested by Ultsch (1978) was almost unaffected even at lethally low pH, and these fish were in this respect similar to *P. tasmanicus*.

The broad pH zone over which respiration of *P. tasmanicus* remains unchanged is associated with a tolerance to a very wide range of external pH, much wider than that tolerated by many other crayfish. For example. *Austropotamobius pallipes* is seriously affected by pH less than 6 (Shaw, 1960), as it starts to lose sodium, and crayfish have been exterminated in regions where acid mine drainage has lowered the pH below 6.5 (Schwartz and Meredith, 1962). Sutcliffe (1978) states that most malacostracans cannot tolerate pH less than 5.7 and at low pH "the osmoregulatory mechanisms break down, with a massive increase in the fluxes of water and ions and a loss of

control over the internal salt and water content. Large-scale drinking of the medium occurs and in many respects the reaction to low pH closely resembles the reactions of freshwater animals exposed to saline media". The freshwater crayfishes as a whole have combined a low integumental permeability to salts with a moderately high permeability to water. They also have active salt transport mechanisms with high affinities for the ions which they transport (Shaw, 1961; Smith, 1976). In view of the ion-depleted environment in which *P. tasmanicus* lives, it may be that this species is even less permeable to salts than is common among the freshwater crayfishes. Low permeability might be expected to restrict the inward migration of hydronium and hydroxyl ions, and their hydrated complexes, as well as reducing the loss of salts. The ion-transporting mechanisms evolved for use in an acid environment must apparently function equally well in an alkaline environment, since laboratory animals are able to survive long periods in alkaline pH never normally experienced. This would mean that there would be no 'stress' on the animal over a wide external pH range, as its internal pH would remain virtually unchanged.

The decrease in VO_2 of *P. tasmanicus* at moderately high pH (i.e. pH above 10.0 at 15°C or above 7.6 at 5°C) is possibly due to a cessation of activity by the crayfish. This response is one commonly observed in *P. tasmanicus* in response to adverse, although not necessarily lethal conditions. The animal appears to be able to suspend all but the most necessary activities, and simply waits for conditions to improve.

The effect of low environmental pH on freshwater animals has become of interest recently as 'acid rain' and acid mine effluents have reduced the pH of many lakes, streams and rivers in the world, especially in north-eastern USA, Scandinavian countries and parts of Ontario in Canada (Dillon *et al*, 1978) as well as Western Europe and South Africa (Bell and Nebeker, 1969). Much of this interest has been focussed on the effects of pH on game fish, with less attention being paid to other fish. *Parastacoides tasmanicus* is far more tolerant to both high and low pH than fish are, as can be seen from Table 3.3 which gives the upper and/or lower pH tolerance limits of a number of species of fish.

Table 3.3 Upper and lower pH tolerance limits of fish*

Species	pH limits	Sources
Brook trout	4.1 - 9.8	Creaser (1930), Dunson and Martin (1973), European Inland Fisheries Advisory Commission (1969), Packer and Dunson (1970)
Rainbow trout	4.5 - 9.5	Jordan and Lloyd (1964), Lloyd and Jordan (1964)
Catostomus commersoni	4.8 -	Beamish (1974)
Guppies	4.75-	Dunson *et al* (1977)
Cheirodon axelrodi	3.35-	" " " "
Hyphessobrycon innesi	3.35-	" " " "
Perch	- 9.2	European Inland Fisheries Advisory
Roach	-10.4	Commission (1969)
Carp	-10.8	"
Pike	-10.7	"
Tench	-10.8	"

* The levels of oxygen, carbon dioxide and other chemicals in the water modify the lethal pH to some extent, so the figures given can only by taken as approximate lethal pH limits.

The survival of freshwater invertebrates in waters with extremes of pH has received less attention than has the survival of fish, but there are some studies with which comparison of the results obtained with *P. tasmanicus* can be made. The pH values at which 50% of the larvae and nymphs of 9 species of aquatic insects (caddisflies, dragonflies, mayflies and stoneflies) survived for at least 30 days ranged from pH 2.45 for *Brachycentrus americanus americanus* to 5.38 for *Ephemerella subvaria* (Bell, 1971). Bell and Nebeker (1969) tested 10 species of aquatic insects and found that the pH values at which 50% died after 96 hours ranged from pH 1.50 for caddisflies to 4.65 for mayflies. The pH range of the *Sialis* genus of alderfly is 2.8 - 8.3 (Tarter and Woodrum, 1972), while species of entomostracan Crustacea have been found in waters with pH from 2.5 to 10.4 (Davis and Ozburn, 1969; Frey, 1971; Lowndes, 1952; Prophet, 1963). It is important to note that although a genus or group of species may have a wide pH range, the range of the individual species is usually much less than the total range.

Although many animals would be excluded from areas with extremes of pH, such as the acid streams and rivers with a pH of less than 3 which occur in South America, or the alkaline lakes with a pH above 10 in East Africa (Johansen and Weber, 1976), most animals have tolerance limits outside the pH ranges experienced by them. Usually some other limiting factor has come into effect long before these extremes are reached (Lawson, 1978).

It is apparent that in comparison with other freshwater invertebrates, the tolerance of *P. tasmanicus* to acid and alkaline pH is quite remarkable.

It is important to realise that although some stages of an animal's life cycle may be tolerant of extremes of pH, other stages

may be much less so. With fish the eggs are especially susceptible (Johansson, Kihlstrom and Wahlberg, 1973). For example, when brook trout eggs were incubated at pH 4.6 only 76% hatched (Trojnar, 1977) and Menendez (1976) showed that prolonged exposure of brook trout eggs to a pH less than 6.5 results in a significant reduction both in egg hatchability and also in growth. Similarly, flagfish *(Jordanella floridae)* need a pH above 6.5 for success in all stages of their life cycle, although the tolerance of the adults is much greater than this (Craig and Baksi, 1977; Ruby, Aczel and Craig, 1977). Adult fathead minnows *(Pimephales promelas)* can survive for at least 13 months at pH 4.5, but at a pH of less than 5.9 all of the eggs that are produced are abnormal (Mount, 1973).

As with fish, tolerance to pH in invertebrates may vary through the life cycle. For example, the midge, *Tanytarsus dissimilis,* requires a pH above 4.0 for the larvae to survive, and a pH above 5.0 for adults to emerge and for eggs to hatch (Bell, 1970). *Daphnia pulex,* although fairly tolerant of a wide range of pH, will only undergo parthenogenesis if the pH is between 7.0 and 8.7 (Davis and Ozburn, 1969).

It is possible that although *P. tasmanicus* in natural conditions never encounter a pH low enough to put them under stress, juveniles or eggs might, if they are less tolerant than adults. However, this is unlikely. Juveniles are found throughout the summer, when the pH of the available water reaches its lowest, and gravid females show no apparent preference for burrow systems different from those occupied by other adults, and yet the hatching success of eggs is high. In normal conditions at least, none of the stages of the life cycle would appear to be in danger from the pH that is encountered. However, it is impossible to say whether or not prolonged exposure of animals to low (or high) pH would impair

growth, feeding or some other activity necessary for successful survival.

The actual cause of death of invertebrates exposed to excessively high or low environmental pH has not been elucidated yet, apart from the work by Shaw (1960) and Sutcliffe (1978), although a reasonable amount of information has been accumulated for fish. The major effects of exposure to low pH, which may lead to the death of fish are

(1) Suffocation due to coagulation of mucus on the gills (Dively *et al*, 1977; Plonka and Neff, 1969; Ultsch and Gros, 1979; Vaala and Mitchell, 1970; Westfall, 1945). This is possibly one reason why Wiebe *et al* (1934) found that at low pH fish are unable to extract enough oxygen from the water for survival except at very high oxygen concentrations. Townsend and Cheyne (1944) found that silver salmon *(Oncorhynchus kisutch)* died from suffocation due to an inability to extract sufficient oxygen from water with a high acid concentration, but they did not identify the reason for this.

(2) Massive loss of sodium (Dively *et al*, 1977; Packer and Dunson, 1970, 1972) and possibly other salts (see Daye and Garside (1976) for the effect of pH on epithelium of the gill lamellae of brook trout).

(3) A drop in blood pH (Packer and Dunson, 1970 ; Packer, 1979) which may have subsequent deleterious effects, such as a decrease in the oxygen carrying capacity of the blood, resulting in asphyxiation (Eddy, 1976; Green and Root, 1933; Root, 1931; Packer, 1979). Some fish are capable of producing haemoglobins that are relatively insensitive to pH effects (Johansen and Weber, 1976), i.e. haemoglobins that have smaller Root and Bohr effects than 'normal' haemoglobins, and these fish probably do

not die from asphyxiation when exposed to lethally low pH.

Lloyd and Jordan (1964) concluded that the cause of death in rainbow trout exposed to high acid concentrations was acidaemia, and Jonas, Sehdev and Tomlinson (1962) found that if these fish were injected with lactic or hydrochloric acid, they died if their blood pH dropped below pH 6.8 - 6.9 but not if the injection of a similar quantity of acid did not lower their blood pH into this range.

The cause of death in fish exposed to high pH is believed to be a (rapid) destruction of the gill and skin epithelium by hydroxyl ions (several authors cited in European Inland Fisheries Advisory Commission, 1969).

In the case of *P. tasmanicus* it seems highly unlikely that suffocation, whether due to mucus coagulation in the gills, or due to reduced oxygen carrying capacity of the haemolymph is the cause of death, since it was shown quite clearly that *P. tasmanicus* exposed to lethally low pH loses large amounts of sodium, whilst potassium losses are no different from those of control animals. Oxygen consumption is not depressed at either the high or low lethal pH. In a variety of biological systems the active transport of sodium and potassium ions is inhibited by low pH (Macan, 1963; Potts and Parry, 1964; Shaw, 1960; Heinz, 1967; Sutcliffe and Carrick, 1973) either because the hydrogen ion concentration may directly affect the transport pump by influencing transport cell metabolism or because the hydrogen ions compete with the sodium ions for active sites on the carrier molecules (Packer and Dunson, 1970). An excessively high or low haemolymph pH would affect the structure of many enzymes and other large molecules and impair their functions.

Many invertebrates are able to cope temporarily with a departure from their normal pH range as long as this departure is not too great or too prolonged. An influx of H^+ ions can be buffered with calcium carbonate which is in high concentration in the haemolymph, exoskeleton and tissues of many invertebrates, as well as in the gastroliths of some crustaceans. Addition of base to the blood can be buffered by the production of H^+ and HCO_3^- ions in the blood by an increase in the carbon dioxide content (Muntwyler, 1968). These mechanisms are, however, only temporary measures, and animals are restricted to regions where the pH can be tolerated for most of the time without resorting to them. Despite the fact that it lives in what may be considered a very inhospitable and acid environment, *P. tasmanicus* can survive successfully. It does not appear to have any special adaptations to live in this environment apart from its apparent impermeability to H^+ ions (or selective excretion of these ions) and the obvious resistance of its epithelium to damage except at very low pH, and when the environment is too acidic, death is associated with a loss of sodium, as in some other animals that are intolerant of extremes of pH. In its normal environment the survival of *P. tasmanicus* is not affected by pH nor is its blood pH or its oxygen consumption.

4. THE EFFECT OF TEMPERATURE AND HUMIDITY ON *Parastacoides tasmanicus*

4.1 Introduction

Parastacoides tasmanicus lives in an area of high rainfall, but it does not always have free water in its burrow. Dry periods do occur, during which the level of the water table may fall below the lowest point of the crayfish burrow, thus requiring the crayfish to survive for days or even weeks without free water.

One of the main dangers facing terrestrial and semi-terrestrial decapods (and indeed most terrestrial animals) is the possibility of desiccation, resulting in death. Herreid (1969a, 1969b) stated that decapods can lose water via a number of routes -

(1) in the faeces,

(2) in the urine,

(3) from the branchial chamber in the form of droplets lost from the chamber itself rather than as water vapour lost from the gills,

(4) as water vapour lost during ventilation of the gills, and

(5) by evaporation from the general body surface.

Other possible sites of water loss are the mouth and anus (as distinct from the faeces). Isopods such as *Armadillidim vulgare* and *Porcellio scaber* lose at least 30% of their total water loss as discharge from the alimentary canal, mainly via the mouth onto the integument (Lindqvist, 1972).

Water loss associated with the defaecation of decapods has received little attention in the literature. *Gecarcinus lateralis* may be able to use its fore-gut as a store for water, especially during ecdysis (Mantel, 1968), but how much of the water can be removed from the food and digestive juices in the alimentary tract is not known. Animals which are well adapted to terrestrial life such as cockroaches, can absorb water from the faeces as necessary. Well-hydrated cock-roaches excrete semi-liquid faeces which are about 80% water, but when these animals are denied water to drink, the water content of the faeces is quickly reduced and discrete faecal pellets are produced. Dehydrated cockroaches also drastically reduce the rate of passage of material through the gut (Tucker 1977).

Table 4.1 Rate of urine production of some decapod crustaceans

	Urine production rate (% body weight/day)	Source
Freshwater crayfish		
Astacus astacus	3.84 - 4.08	Hermann (1931), Scholles (1933)
" "	8.22	Bryan (1960)
Procambarus clarkii	5.28	Lienemann (1938)
Freshwater amphibious crabs		
Potamon niloticus	0.05 - 0.6	Shaw (1959)
Potamon edulis	0.58	Harris (1975)
Terrestrial crabs		
Cardisoma guanhumi	1.89	Harris (1977)
Gecarcinus lateralis		
on wet sand	10.47	" "
on dry sand at 55% humidity	2.15	" "

Crabs such as *Cardisoma*, *Gecarcinus* and *Ocypode* reduce urine production by changing from ammonotelism when water is plentiful to uricotelism when water is scarce (Bliss, 1968; Bliss and Mantel, 1968; Edney, 1977). *Cardisoma guanhumi*, *Gecarcinus lateralis* and *Ocypode cordimana* all form deposits of uric acid in their pericardial sacs and/or viscera. Presumably these deposits are disposed of when the animals moult, as they are absent immediately after ecdysis (Bliss, 1968; Bliss and Mantel, 1968; Gifford, 1968; Rao, 1968).

Some semi-terrestrial crabs always have their branchial cavities partly filled with water (Edney, 1960; Wolvekamp and Waterman, 1960), while many fully terrestrial crabs do not. The rate of loss of this water is unknown and probably extremely variable. Herreid (1969a) did not observe any water discharge from the gill chambers of 11 species of brachyuran crabs while they were kept out of water.

Terrestrial and semi-terrestrial crabs normally have fewer gills than more aquatic species (Veerannan, 1974). More importantly, the gill volume and surface area per unit weight are also decreased, thus reducing evaporative water loss (Gray, 1957; Vernberg and Vernberg, 1968). This reduction is possible because of the much greater percentage of oxygen in air than in water. Of course the gills need some support, and so some terrestrial species have the lamellae of the gills strengthened with chitinous ridges, which presumably keep them erect and functional in the air (Harms, 1932). Diaz and Rodriguez (1977) have studied the branchial chambers of terrestrial crabs, and their paper should be referred to for a fuller coverage of this topic. Collapse of the gills is not necessarily disastrous, as *Potamonautes* sp. can live in air for some time even though the gills collapse, provided that the gills are kept moist (Edney, 1960), although it is possible that these animals have "lungs" to aid respiration (see below).

Some of the more terrestrial species of crabs do not use their gills much for respiration. Instead the epithelial membrane that lines the branchial chambers has evolved into a highly vascularised and folded 'lung' (Bliss, 1968; Vernberg and Vernberg, 1968) and the gills are restricted to a small portion of the branchial chamber. The Australian arid-zone crab, *Holthuisana transversa*, has even gone so far as to evolve a lung-like ventilatory action with a tidal rhythm (Greenaway and Taylor, 1976).

Whether the respiratory site is represented by gills or by a vascularised epithelial membrane, it must be kept moist if it is to function effectively, and it is therefore the site of some water loss, although the water loss from terrestrial species is usually lower than that from aquatic species.

Those land arthropods that are most successful in dry regions, such as insects, arachnids, millipedes and centipedes, possess a waxlike layer in their epicuticle which effectively prevents rapid evaporation from the integument (Edney, 1960, 1977). Crustaceans do not possess such a layer and therefore lose water from the integument. However, the ratio of this integumental loss to the loss from the branchial chambers (as water vapour lost during ventilation of the gills) varies. Herreid (1969b) claimed that more than 50% of the evaporative water loss of land and aquatic crabs is from the integument, and that the permeability of the exoskeleton of aquatic species is greater than that of terrestrial species. Dandy and Ewer (1961) calculated that at 20°C and 20% relative humidity (R.H.) the amphibious crab, *Potamon depressus*, loses approximately 41% of its water loss from the integument. They also suggested that a change in the permeability of the exoskeleton might occur as it dried. Ahsanullah and Newell (1977), on the other hand, concluded that at 15°C and 55% R.H., *Portunus marmoreus* and *Carcinus maenas* lost most water from the respiratory surfaces and branchial integument.

When crabs and crayfish are out of water, it is the rate of water loss (R.W.L.) and the amount of water that they can lose before they are irreparably damaged, that determines how long they will survive. In general the evaporative water loss of crabs from terrestrial environments is less than that of species from aquatic habitats (Edney, 1962; Herreid, 1969a) and the water loss that terrestrial species can withstand is usually higher than that which less terrestrial species can tolerate (Bliss, 1968; Dandy and Ewer, 1961; Greenaway and MacMillen, 1978; Herreid, 1969a; Young, 1978).

There are sometimes other problems associated with water loss apart from body dehydration. For example, if the respiratory surfaces

of woodlice, namely the pleopods and integument,dry out, the animals may die of asphyxiation (Edney and Spencer, 1955). There is the possibility that this could happen to *Parastacoides tasmanicus*, although it is now known that when this species is removed from water its aerial respiration rate is *initially* equal to its aquatic rate (see Chapter 7).

As stated earlier, *P. tasmanicus* does have to survive periods without free water in its burrow. However, even in 'dry' crayfish burrows it is unlikely that the R.H. would drop considerably below 100%, and *P. tasmanicus* is believed not to venture out of its burrow except at night when the R.H. is high. Of course this behaviour is itself an adaptation that would prolong the survival of the crayfish in dry conditions, although it is probable that it evolved as a mechanism for escaping predators and high summer temperatures rather than as a water conserving mechanism. It would seem appropriate to measure the factors affecting the survival of *P. tasmanicus* out of water, i.e. R.W.L. and lethal water loss (L.W.L.), at high humidities rather than at low humidities which the animal would never normally encounter. It is possible that summer-adapted animals differ from winter-adapted animals in their ability to survive out of water, and this difference has also been investigated.

No distinctions were made between the two subspecies of crayfish found in the collection area, i.e. *P. tasmanicus tasmanicus* and *P. tasmanicus insignis*. As stated in Chapter 2, there is little difference in morphology and habitat selection between the two subspecies, so it was assumed that their ability to withstand dehydration was similar. Unfortunately insufficient animals were available at the end of this study to allow this assumption to be tested. The third subspecies, *P. tasmanicus inermis* possibly may differ from the other two in its ability to survive dehydration, as it is found in drier areas, but this subspecies was not available in the study area.

4.2 Materials and methods

4.2.1 Respiratory appendages of *Parastacoides tasmanicus*

Preserved specimens of *Parastacoides tasmanicus* were examined to determine the number and types of gills present and to determine if there were any obvious morphological adaptations to an aerial existence.

4.2.2 Water loss of crayfish kept on damp filter paper

The survival of *P. tasmanicus* on a damp substrate at 15° and 20°C (representing normal and high summer burrow temperatures) was determined, using adult and juvenile crayfish, as follows. Adult specimens were washed with filtered button grass water, drained and gently shaken, then wiped with tissue paper to remove water from the integument and appendages. The animals were then placed in individual glass jars that had damp filter paper on the bottoms, sides and lids. Juvenile crayfish were washed and dried and placed in individual 25 mL beakers that had damp filter paper in their bottoms. The beakers were placed in a large container with some water in it to keep the R.H. at about 100%. All of the containers were covered with black plastic to minimise visual disturbance of the animals and placed in constant temperature rooms at the appropriate temperatures. The animals were each weighed every second day, any weight loss being assumed to be due to moisture loss.

4.2.3 Water loss of crayfish at 99-100% R.H.

A number of crayfish were kept at 99-100% R.H. at 15° and 20°C but without any access to moisture. These animals were washed and dried, and each one was placed in a weighed plastic (Duranol) vial. They were confined by plastic mesh held over the mouths of the vials with rubber bands. The vials were placed over water in a desiccator and were weighed each day to determine the weight losses of the

animals. Measurements of the weights of some of the animals were continued for several days after death.

4.2.4 Water loss of crayfish at 93% R.H.

The rate of water loss of *P. tasmanicus* at 93% R.H. was measured by placing washed and dried animals in Duranol vials (as above), and then putting them over a potassium hydroxide solution of the appropriate strength (approximately 1·8 mol KOH/L H_2O in solution, according to Solomon (1951)) in desiccators. These animals were also kept in constant temperature rooms at 15° and 20°C and weighed daily until several days after death.

There was deliberately no forced air circulation in any of the experiments. In a crayfish burrow it is unlikely that there would be much air movement, and consequently a humidity gradient would be expected to form around an animal. The same conditions would occur with the laboratory techniques employed. This would mean that the R.H. at the surface of the animal would probably be higher than the nominal R.H. but it was considered that experimental results obtained under such conditions would give a more realistic measure of the survival time and R.W.L. of animals in 'natural' conditions than if a technique employing continuous air currents over the animals had been used.

4.2.5 Comparison of 15°C-acclimated and 5°C-acclimated crayfish at 93% R.H.

Animals that had been caught in winter and maintained in the laboratory at 5°C for 2 months were considered separately from animals that had been caught during summer and kept at 15°C for 2 months. Both groups of animals were tested at 15° and 20°C at 93% R.H. and the results were tested with a small-sample Student's t-test to see if the differences in response were significant.

4.2.6 Partitioning of water loss of *Parastacoides tasmanicus*

An attempt was made to determine what proportion of the water loss of dehydrating crayfish was integumental. Control animals in 93% R.H. and 70% R.H. in desiccators at 15°C were weighed daily. Other animals in which the entire integument was covered with petroleum jelly, in order to eliminate or at least greatly reduce water loss from that source, were also kept in 93% and 70% R.H. at 15°C, and weighed daily to determine their water loss.

The experiment at 93% R.H. was continued for 20 days, and the experiment at 70% R.H. was continued for 12 days.

4.2.7 Cause of death of *Parastacoides tasmanicus* kept out of water

There are several procedures available that could be used to determine whether *P. tasmanicus* dies from asphyxiation or dehydration when kept in air. Firstly, if there is normally a large water loss across the integument, then the prevention of this water loss should mean that although treated animals (i.e. animals with their integumental water loss greatly reduced by a covering of petroleum jelly, for example) dying from dehydration would live longer than untreated animals, they would still die with the same total water loss. If, however, the petroleum-jelly coated animals die from asphyxiation then they would be expected to die at the same time as untreated animals, but the amount of water lost before death should be less than that lost by untreated animals, which also lose water from the integument.

Secondly, if the cause of death is asphyxiation, then according to Dandy and Ewer (1961) it would be expected that animals kept at a high humidity would asphyxiate and die with a lower total water loss than animals kept at a lower humidity. For this second test, animals were exposed to 70% R.H. and their survival time, L.W.L. and

R.W.L. were measured. These results were used in conjunction with results from the tests at 93% and 100% R.H.

The results from these two experimental techniques should make it possible to determine whether *P. tasmanicus* dies from dehydration or asphyxiation.

What might appear to be a useful method of testing to see if an animal is dying from asphyxiation, namely by measuring the oxygen consumption of hydrated and dehydrated animals, is in fact not very useful at all. *Parastacoides tasmanicus* usually becomes inactive in adverse conditions, and therefore is likely to reduce its oxygen consumption when placed in dehydrating conditions, even if it is not dying from asphyxiation. This view is supported by the observation of MacMillen and Greenaway (1978) that the Australian arid-zone crab, *Holthuisana transversa,* reduces its oxygen consumption by up to 90% when placed in dehydrating conditons, and this reduction is certainly not due to asphyxiation.

4.3 Results

4.3.1 Respiratory appendages of *Parastacoides tasmanicus*

Parastacoides tasmanicus has 17 or 18 pairs of gills plus a pair of respiratory epipodites associated with the first thoracic segment. The gill formula is given in Table 4.2. The gills totally fill the branchial chamber, and there is no evidence of any excessive vascularization of the epithelial lining, or obvious increase in structural support of the gills, as occurs in some terrestrial crabs. The crayfish therefore presumably has to rely on its gills to supply its oxygen requirements. When the animal is removed from water a considerable amount of water is trapped within the branchial chambers, but much of this soon drains out. The scaphognathite continues beating for a time after the animal is removed from water, but it eventually stops when it becomes dehydrated. This does not seem to affect the survival of the crayfish.

Table 4.2 The gill formula of *Parastacoides tasmanicus*

Gill type	Thoracic segment							
	I	II	III	IV	V	VI	VII	VIII
Podobranch	-*	+	+	+	+	+	+	-
Arthrobranch-anterior	-	+	+	+	+	+	+	-
Arthrobranch-posterior	-	-	+	+	+	+	+	-
Pleurobranch	-	-	-	-	-	-	-	+or-

* = respiratory epipodite - = gill not present + = gill present

4.3.2 Urinary and faecal water loss of *Parastacoides tasmanicus*

Urinary and faecal water loss of *P. tasmanicus*, although not directly measured, must have been very low. At 99-100% R.H. at 15°C, with no access to any source of water, the rate of water loss of *P. tasmanicus* ranged between 0.36% body weight/day for a 4.57 g

crayfish to 2.04% body weight/day for a 0.14 g crayfish. Furthermore, at all temperatures and humidities the R.W.L. did not vary noticeably after death (see Figures 4.1a, 4.1b, 4.2a, 4.2b, 4.3a and 4.3b), so the urinary and faecal losses were clearly extremely low. What little faeces were produced were retained in the body for longer than normal, perhaps indicating a reduction in metabolic activity. At 15°C food normally passes through the digestive tract of *P. tasmanicus* within 1 to 3 days, but animals out of water were still producing faeces after 4 weeks without food. The crayfish kept their abdomens curled up, and the faeces produced were retained inside the folded abdomen. This would almost certainly have greatly reduced the water loss from the pleopods and soft underside of the abdomen. When they are in water *P. tasmanicus* usually keep their abdomens extended.

4.3.3 Water loss of crayfish kept on damp filter paper

Of the 24 animals kept on damp filter paper, 12 at 15°C and 12 at 20°C, only 3 died before the experiment was terminated after 550 hours. One of the animals that died was just starting to moult, and apparently died because it was unable to do so while out of water. It had lost only 6% of its initial weight, so its death was most unlikely to have been due to dehydration. The cause of death of the other two animals is unknown, but was not believed to be dehydration, as they had lost only 3-4% of their initial weight at death. Table 4.3 shows the weight loss and R.W.L. of the remaining crayfish. As can be seen from the large standard errors, there was a great deal of variation in total weight loss and R.W.L. However, none of the animals lost more than 10% of their initial weight over the 550 hours. Surprisingly, it was the smallest animals that lost the least weight, and 5 of the smaller animals, which ranged in size from 0.0421 to 0.79 g, actually gained weight.

Figure 4.1a Decrease in weight of 6 *Parastacoides tasmanicus* exposed to 99-100% relative humidity, at 15°C.

Symbol	Initial weight of crayfish (g)
●	4.57
■	3.61
▽	2.91
▲	1.46
○	0.82
□	0.14

Solid lines indicate weights of live animals, broken lines indicate weights of animals after their deaths.

Figure 4.1b Decrease in weight of 6 *Parastacoides tasmanicus* exposed to 99-100% relative humidity, at 20°C.

Symbol	Initial weight of crayfish (g)
●	3.95*
■	3.45*
▽	1.97*
▲	1.40*
○	0.61
□	0.10

*Still alive when the experiment was terminated after 840 hours.

Solid lines indicate weights of live animals, broken lines indicate weights of animals after their deaths.

Figure 4.1a

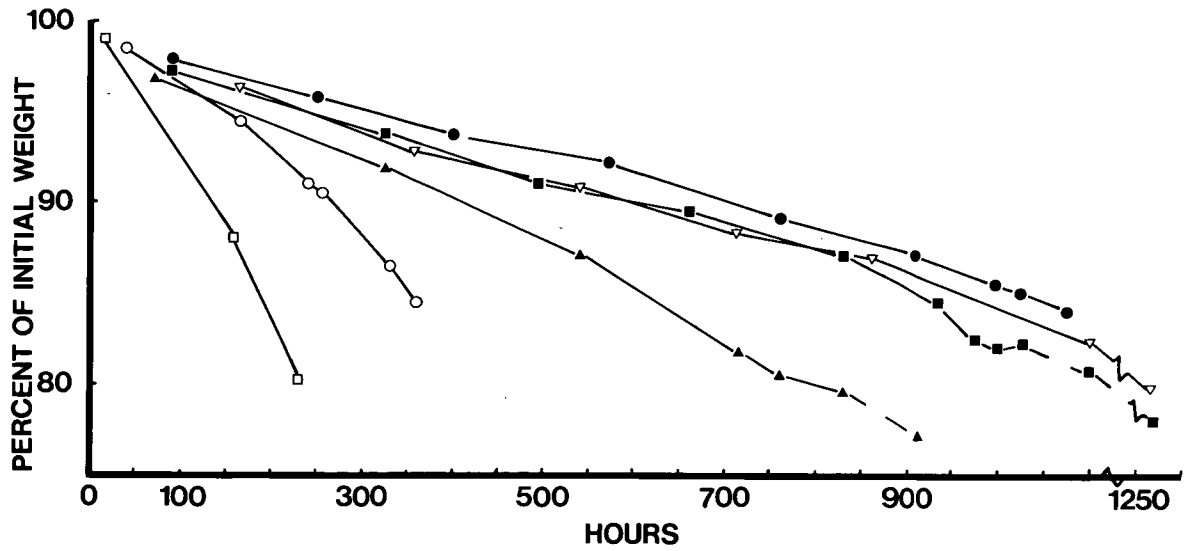


Figure 4.1b

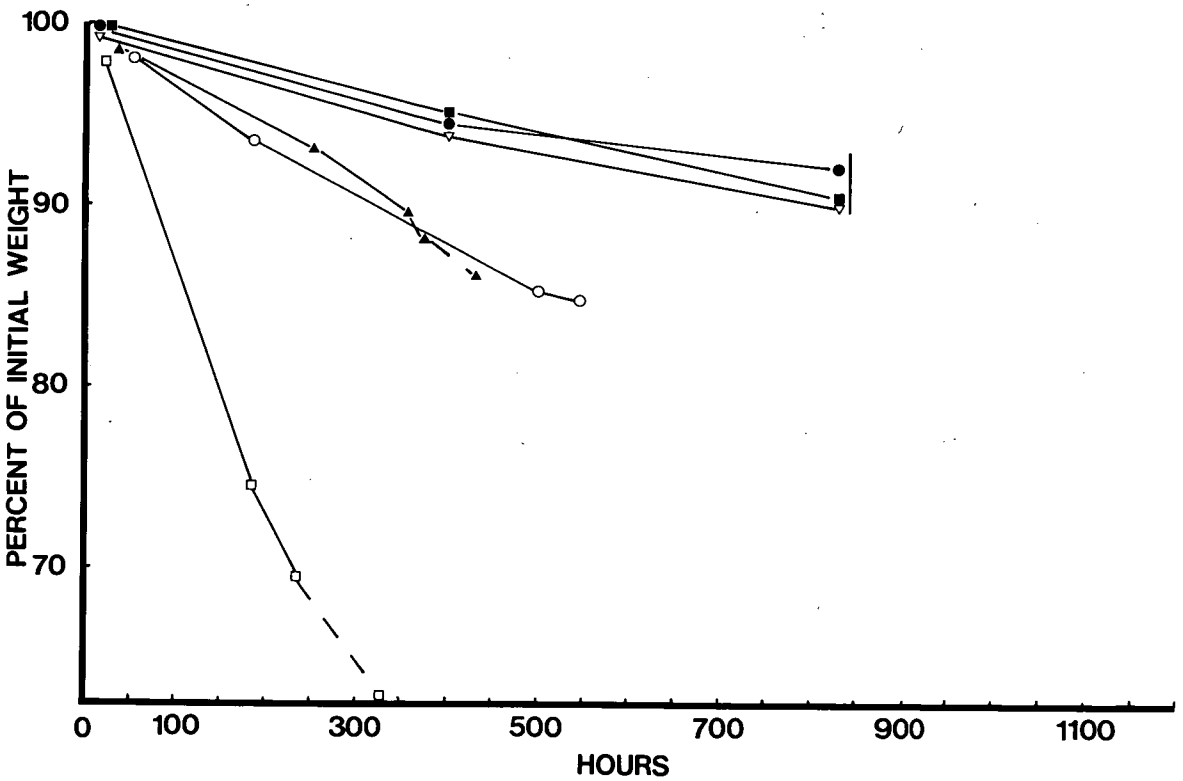


Figure 4.2a Decrease in weight of 6 *Parastacoides tasmanicus* exposed to 93% relative humidity, at 15°C, after acclimation to 15°C.

Symbol	Initial weight of crayfish (g)
●	3.04
■	2.19
▽	2.18
▲	1.47
○	1.13
□	0.38

Solid lines indicate weights of live animals, broken lines indicate weights of animals after their deaths.

Figure 4.2b Decrease in weight of 6 *Parastacoides tasmanicus* exposed to 93% relative humidity, at 20°C, after acclimation to 15°C.

Symbol	Initial weight of crayfish (g)
●	3.40
■	2.89
▽	1.93
▲	1.87
○	1.76
□	0.39

Solid lines indicate weights of live animals, broken lines indicate weights of animals after their deaths.

Figure 4.2a

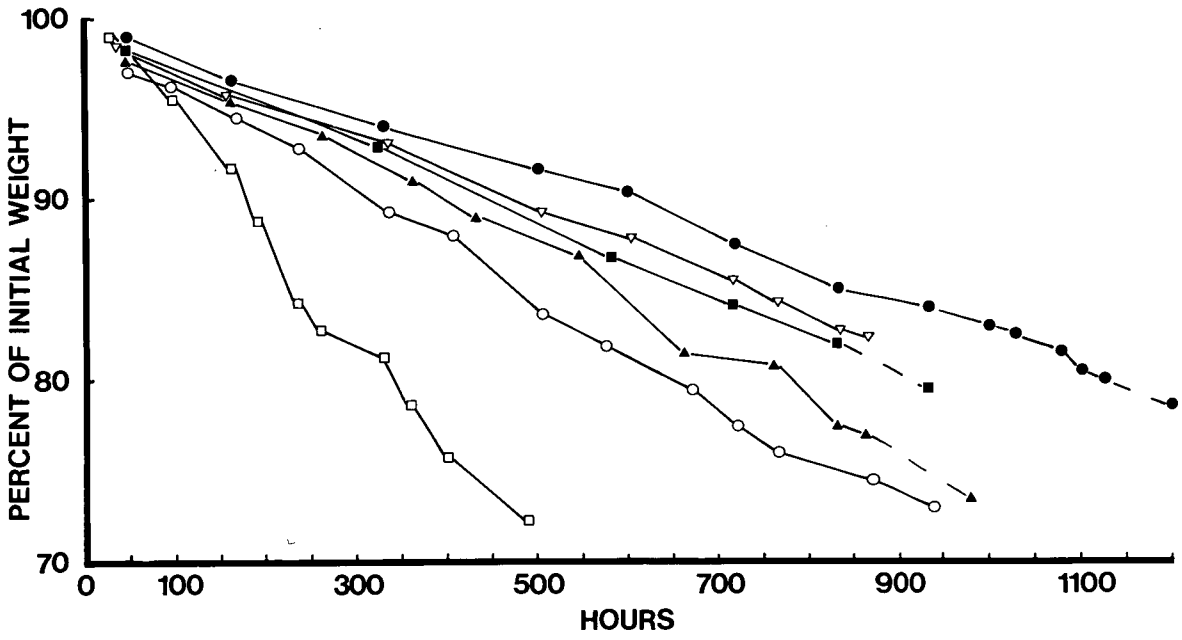


Figure 4.2b

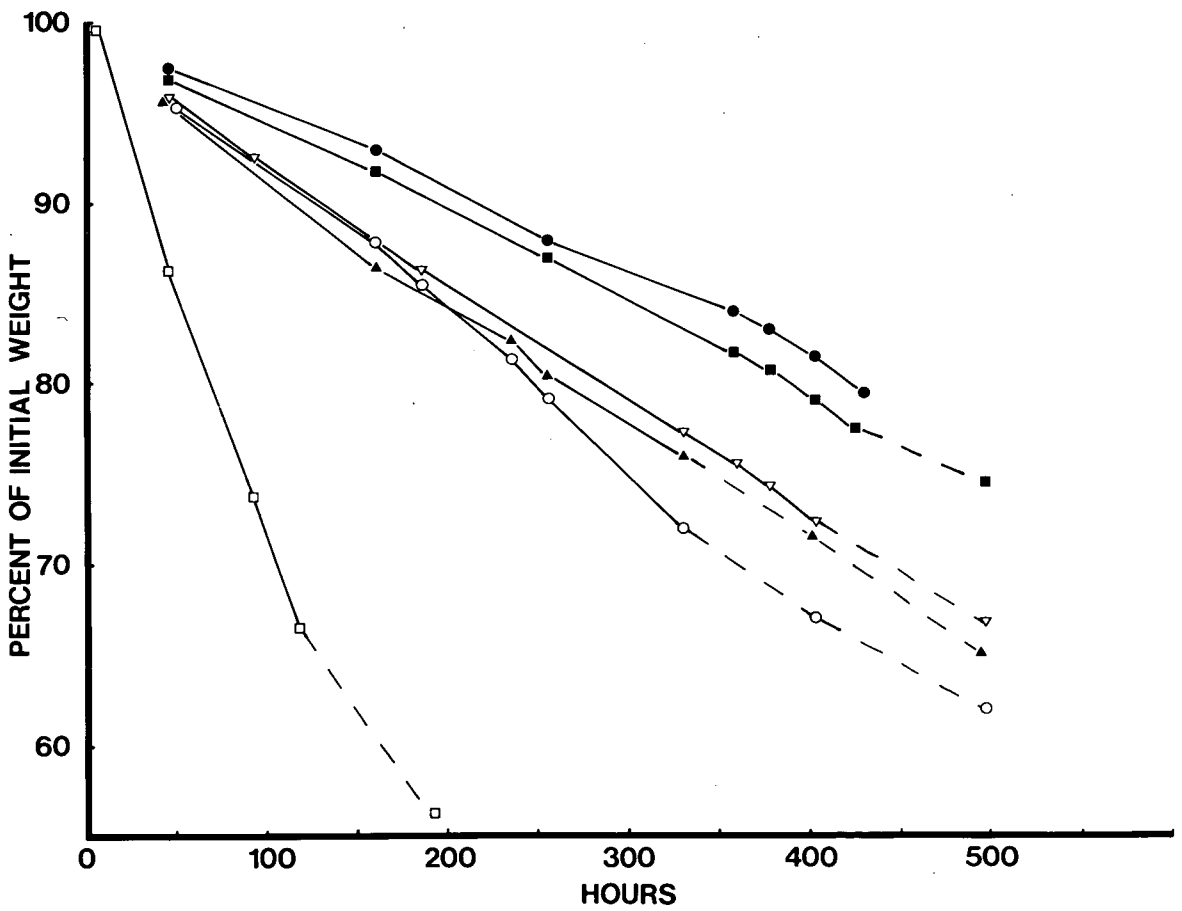


Figure 4.3a Decrease in weight of 6 *Parastacoides tasmanicus* exposed to 93% relative humidity, at 15°C, after acclimation to 5°C.

Symbol	Initial weight of crayfish (g)
●	3.95
■	2.73
▽	2.31
▲	2.07
○	1.93
□	1.71

Solid lines indicate weights of live animals, broken lines indicate weights of animals after their deaths.

Figure 4.3b Decrease in weight of 6 *Parastacoides tasmanicus* exposed to 93% relative humidity at 20°C, after acclimation to 5°C.

Symbol	Initial weight of crayfish (g)
●	3.45
■	3.02
▽	2.68
▲	2.65
○	2.62
□	2.09

Solid lines indicate weights of live animals, broken lines indicate weights of animals after their deaths.

Figure 4.3a

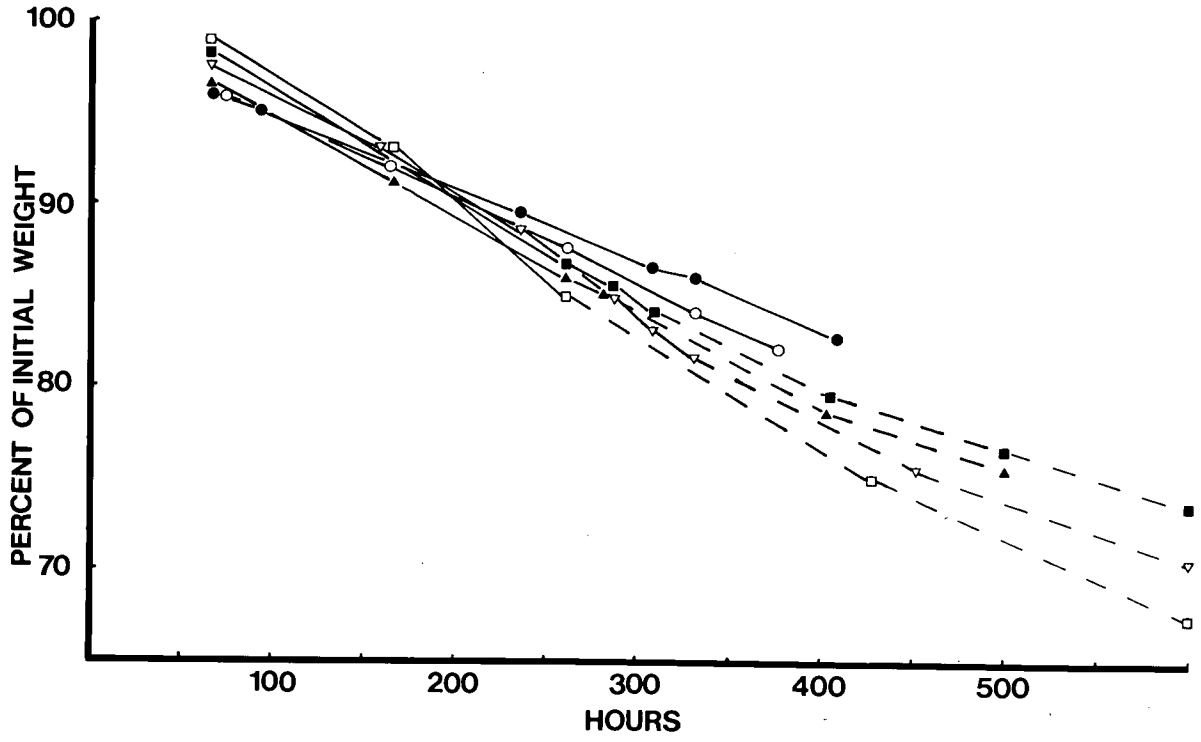
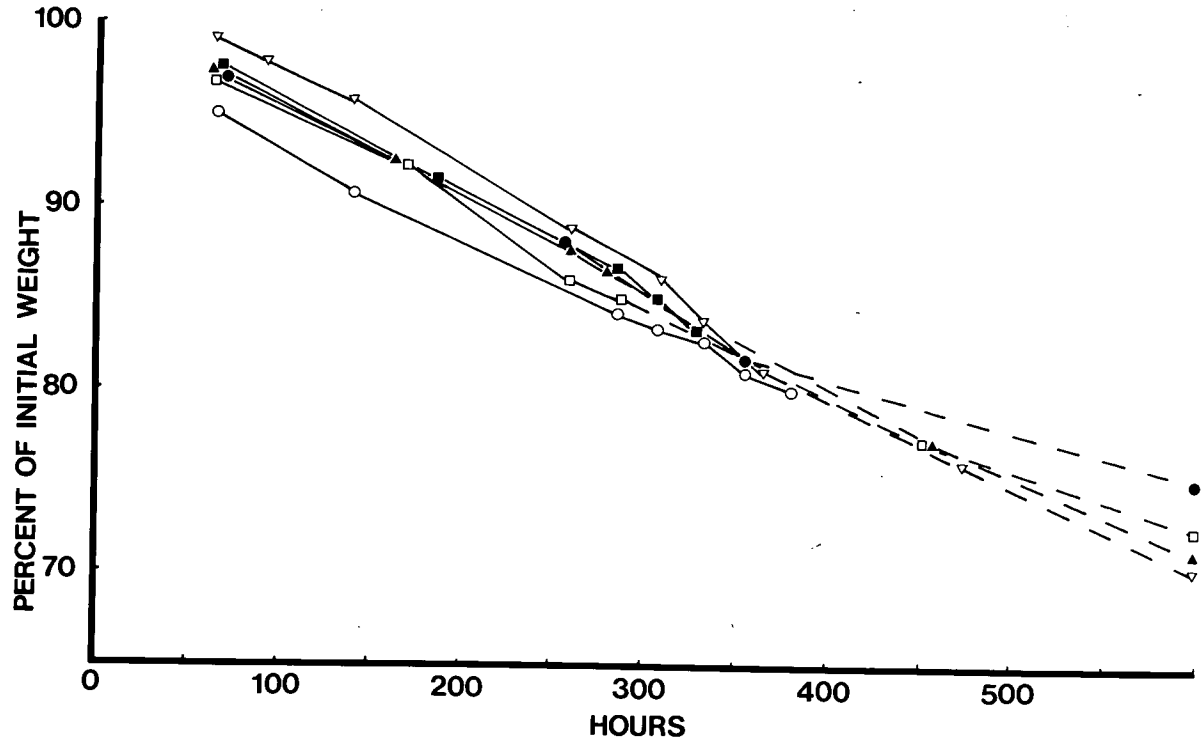


Figure 4.3b



These animals could apparently take up water from very small water droplets which condensed on the walls of their containers; larger animals were unable to do this. Some of the larger animals did, however, eat damp filter paper and may have reduced their water loss by absorbing water from the gut.

Table 4.3 Weight loss of crayfish kept on damp filter paper for 550 hours

Temperature (^{o}C)	15	20
Initial weight of animals* (g)	1.17 ± 0.35 (12)	1.65 ± 0.54 (9)
Total water loss* (percent initial body weight)	0.98 ± 1.24 (12)	2.33 ± 1.76 (9)
R.W.L.* (Percent body weight lost per hour)	0.0018 ± 0.0023 (12)	0.0042 ± 0.0032(9)

* Mean ± S.E. (N)

4.3.4 Water loss of crayfish at 99-100% R.H.

The change in weight in 99-100% R.H. of 15^{o}C-acclimated crayfish with no access to free water, at 15^{o} and 20^{o}C, is shown in Figures 4.1a and 4.1b respectively. It is obvious that in these circumstances the smaller animals lose water at a greater rate than do larger animals. The loss of weight with time is almost linear, with no obvious change of slope occurring after the death of the animals, thus indicating that live animals have no more control over their R.W.L. than do dead ones, and also that water loss from the gills does not decrease after death.

The 20^{o}C experiment was terminated after 5 weeks because it was thought that the animals could have been starting to suffer from starvation. The 15^{o}C experiment was continued until all of the animals had died, even though one animal survived for 7½ weeks. Starvation may have been a contributing factor in the death of these animals, but this is unlikely. The weight loss of these

animals was approximately the same as the weight loss of animals that died after a shorter exposure to a lower R.H. (see Table 4.4.).

Table 4.4 L.W.L., R.W.L. and survival times of crayfish exposed to various R.H. at 15°C.

R.H. %	70	93 (15° C-acclim.)	93 (15° C-acclim.)	99-100
Initial weight* (g)	.2.56 ±	0.96(5). 1.73 ±	0.38(6). 2.45 ±	0.33(6). 2.25 ±0.70(6)
L.W.L.* (%)	.18.52±	0.71(5). 20.62±	1.08(6). 16.61±	0.63(6). 18.33± 0.86(6)
Survival time* (h)	.248 ± 21	(5). 801 ± 102	(6). 327 ± 23	(6). 799 ± 72 (6)
R.W.L.* (%/h)	.0.078±	0.005(5). 0.030±0.006	(6). 0.052±0.002(6).	0.034±0.011(6)

*Mean ± S.E. (N)

4.3.5 Comparison of 15°C-acclimated and 5°C-acclimated crayfish at 93% RH

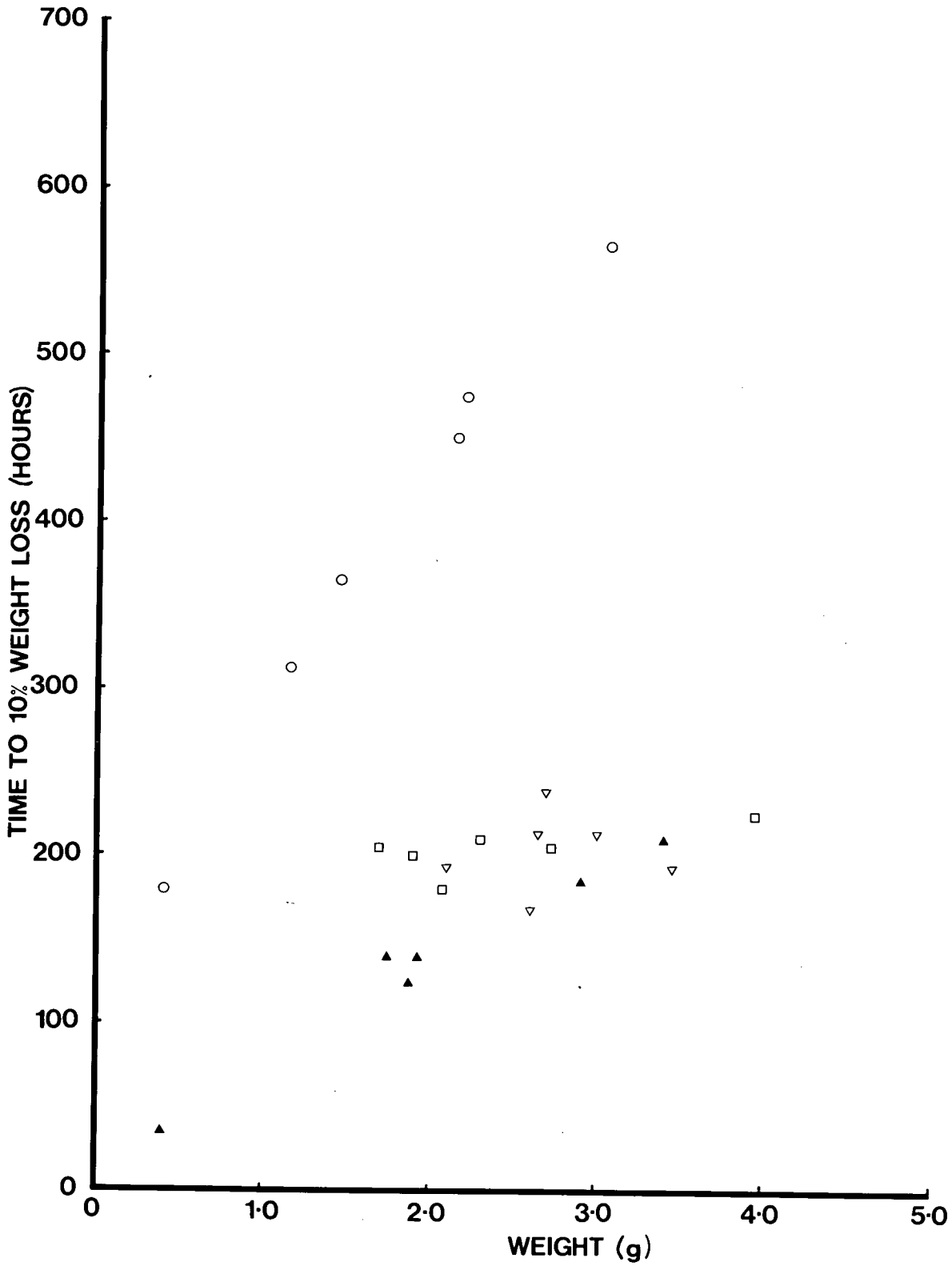
Figures 4.2a and 4.2b show the loss of weight of 15°C-acclimated (summer) crayfish at 15° and 20°C (at 93% R.H.) respectively. Figures 4.3a and 4.3b show the results obtained by repeating the experiments with 5°C-acclimated crayfish (i.e. winter animals). The most noticeable differences between the two sets of graphs are the differences in survival times of animals of similar weights. 15°C-acclimated crayfish survive longer than do 5°C-acclimated crayfish at 15°C but not at 20°C (see Table 4.5). This difference in survival times is associated with a difference in the L.W.L. at both temperatures, and by a change in the R.W.L. at 15°C. It is interesting to note that the 5°C-acclimated animals did not survive any longer at 15°C than they did at 20°C. There was no difference between the L.W.L., R.W.L. and survival time of the two groups.

The difference between the 15°C-acclimated and 5°C-acclimated animals can be shown graphically. Figure 4.4. shows the relationship between the weight of animals treated in various ways and the time to 10% weight loss. When the times required for animals to lose 10% of their body weight are compared, there appears to be little difference between 5°C-acclimated animals tested at 15° and 20°C, and 15°C-acclimated animals tested at 20°C, whilst

Figure 4.4 Time to 10% weight loss for *Parastacoides tasmanicus* exposed to differing test and acclimation temperatures, at 93% relative humidity.

Symbol	Test temperature (°C)	Acclimation temperature (°C)
○	15	15
▲	20	15
□	15	5
▽	20	5

Figure 4.4


700
600
500
400
300
200
100
0
TIME TO 10% WEIGHT LOSS (HOURS)
0
1·0
2·0
3·0
4·0
5·0
WEIGHT (g)

15°C-acclimated animals at 15°C generally take much longer to lose this proportion of their initial weight. However, an analysis of the regression of 'time to 10% weight loss' against 'weight' (F-test for difference between two regression coefficients) shows that there is a significant difference ($P < 0.01$) between regression coefficients of all of the treatments with the exception of the 5°C-acclimated animals at 15° and 20°C. These statistical differences are primarily caused by a lack of data for low weight animals acclimated at 5°C. If these data were available it is likely that there would no longer be a statistical difference between the 5°C-acclimated animals and the 15°C-acclimated animals tested at 20°C.

Table 4.5 Comparison of 15°C-acclimated and 5°C-acclimated crayfish in 93% R.H. at 15° and 20°C.

	15°C-acclimated		5°C-acclimated		t-test
15°C test					
Initial weight* (g)	1.73 ± 0.38	(6)	2.45 ± 0.33	(6)	No sig. diff.
L.W.L.* (%)	20.62 ± 1.08	(6)	16.61 ± 0.63	(6)	$P < 0.01$
R.W.L.* (%/h)	0.030 ± 0.006	(6)	0.052 ± 0.002	(6)	$P < 0.01$
Survival time* (h)	801 ± 102	(6)	327 ± 23	(6)	$P < 0.01$
20°C test					
Initial weight* (g)	2.04 ± 0.42	(6)	2.75 ± 0.19	(6)	No sig. diff.
L.W.L.* (%)	26.53 ± 2.00	(6)	17.24 ± 1.00	(6)	$P < 0.01$
R.W.L.* (%/h)	0.102 ± 0.037	(6)	0.052 ± 0.001	(6)	No sig. diff.
Survival time* (h)	339 ± 48	(6)	336 ± 17	(6)	No sig. diff.

*Mean ± S.E. (N)

4.3.6 Site of water loss in *Parastacoides tasmanicus*

The results of the experiment designed to investigate the partitioning of water loss between the integument and gills of *P. tasmanicus* show no significant differences between control animals and test (petroleum jelly-coated) animals at either 93% R.H. or 70% R.H. (see Table 4.6). These results suggest that water loss across the integument is very slight, and that most of the water loss of *P. tasmanicus* is through the branchial chambers though it is not possible to say whether the gills or the branchial lining form the major site of loss.

Table 4.6 Comparison of water lost by animals with and without their integument covered with petroleum jelly, at 90% and 70% R.H. at 15°C.

93% R.H.	Control	Test	t-test
Initial weight* (g).	4.68 ± 0.81(6).	4.71 ± 0.82(6).	No sig.diff.
Weight loss in 20 days* (%)	.13.92 ± 0.59(6).	15.04 ± 0.85(6).	No sig.diff.

70% R.H.	Control	Test	t-test
Initial weight* (g).	6.46 ± 0.47(6).	6.08 ± 0.24(6).	No sig.diff.
Weight loss in 12 days* (%)	. 7.98 ± 0.27(6).	7.25 ± 0.23(6).	No sig.diff.

* Mean ± S.E. (N)

4.3.7 Cause of death of *Parastacoides tasmanicus* kept out of water

As *P. tasmanicus* does not lose any measurable amount of water across the integument, it is not possible to see if it dies from asphyxiation or desiccation by determining and comparing the L.W.L. of control animals with animals with restricted water loss. Table 4.4 shows that the L.W.L. of animals which die at a high R.H. is no less than that of animals which die at a lower humidity. In view of the arguments proposed in Section 4.1 the results suggest that the cause of death of the crayfish is not asphyxiation.

4.4 Discussion

Although *P. tasmanicus* has a slightly reduced number of gills compared to some of the decapods, e.g. *Homarus* has 20 pairs of gills (Barnes, 1963), it is unlikely that this is an adaptation for respiring in air. Barnes (1963) reported that other decapods such as the snapping shrimp, *Alpheus*, the swimming crab, *Callinectes*, and the pea crab, *Pinnotheres*, (no species names given) with 6, 8 and 3 pairs of gills respectively all have reduced gill numbers compared to many other decapods, and yet they are all purely aquatic. The Tasmanian stream-dwelling crayfish, *Astacopsis fluviatilis* and *A. tricornis*, both have 21 pairs of gills, plus a pair of respiratory epipodites on the first thoracic segment (R. Swain, unpublished data). However, the burrowing species *Geocharax falcata*, *Engaeus leptorhynchus*, *E. cunicularius* and *E. fossor*, some of which are even more terrestrial than *P. tasmanicus*, also have 21 pairs of gills, some with and some without the epipodite. It is worth noting that Newcombe (1970), who worked at low humidities (and whose results are therefore not discussed in more detail), found that at 21 - 23% R.H. and 12^{o}C a 10.1 g *P. tasmanicus* survived almost 150 hours, while Suter (1975) found that a 10.3 g *Engaeus fossor* only survived 115 hours at 24% R.H. and 15^{o}C. A 4.9g *P. tasmanicus* also survived much longer than a 4.5 g *E. fossor* in similar conditions, so the reduced gill number (or more importantly the reduced gill surface area, assuming that the two are positively related) may serve some purpose in reducing the R.W.L. of *P. tasmanicus* in air.

It is not really surprising that *P. tasmanicus* has no apparent morphological adaptations for respiring in air. Most of the year it is required to survive in water, and mechanisms that allow efficient aerial respiration are often poorly suited to aquatic respiration. Many terrestrial and semi-terrestrial crabs can drown if they are held under water (Bliss, 1968). The semi-terrestrial crab, *Cardisoma guanhumi*, which, like *P. tasmanicus* spends much of its time in a burrow containing poorly oxygenated water, does not have

nearly as reduced a gill surface area to body volume ratio as the crab, *Gecarcinus lateralis*, which lives in a burrow without free water in it (Bliss, 1968). In addition, *C. guanhumi* also possesses a haemocyanin with a high oxygen affinity while *G. lateralis* has a haemocyanin with a low oxygen affinity. *G. lateralis* has a respiratory system that is adapted for a totally terrestrial life whereas *C. guanhumi* has apparently reached a compromise between aquatic and terrestrial respiration. No figures are available on the surface area of the gills of *P. tasmanicus*, nor on the affinity of its haemocyanin, but like *C. guanhumi* it can respire adequately both in poorly oxygenated water and out of water, at least for some considerable time (see Chapter 7). It has been found that the copper ion content of the haemolymph of *P. tasmanicus tasmanicus* is almost 3 times as high as that of *P. tasmanicus inermis* (R. Swain, pers. com.). It could be suggested that this is an adaptation which allows *P. tasmanicus tasmanicus* to respire in poorly oxygenated water not normally encountered by *P. tasmanicus inermis*. The more terrestrial *P. tasmanicus inermis* is found in drier conditions than *P. tasmanicus tasmanicus* and hence probably spends much more of its time respiring in air; consequently it may not need as high a copper (and hence haemocyanin) content as *P. tasmanicus tasmanicus*. However, the copper ion content of the *Astacopsis* species are similar to that of *P. tasmanicus tasmanicus*, and these species are very intolerant of low oxygen levels. It is obvious that much more information is required before the situation can be adequately explained.

The adaptation of crabs to existence in freshwater has been shown by Shaw (1959, 1961) to have involved a lowering of body-wall permeability to both salts and water, with the production of a small volume of urine isotonic to the blood. It is probable that an extrarenal site of water excretion also exists (Potts and Parry, 1964; Shaw, 1959; Thompson, 1970). On the other hand freshwater crayfishes seem to have combined a low integumental permeability to salts with a moderately high permeability to water, and have coupled this

with an active salt-transport system both integumental and nephridial, so that they are able to produce a hypotonic urine, and hyper-regulate to maintain a blood concentration about half that seen in freshwater crabs (Shaw, 1961; Smith 1976). This explains the low rate of urine production of *Potamon niloticus* and other freshwater crab species, and the much higher urine production rates observed in *Astacus astacus* and other freshwater crayfishes. Ten species of freshwater crayfish that have had their urine production measured, are reported to have flow rates between 3% and 8.2% body weight per day (see Wong, 1972). *Paranephrops zealandicus*, the only parastacid crayfish so far tested, has a flow rate of 5.5% body weight per day. The urine flow rate of *P. tasmanicus* in water is at present unknown. It would be expected that the urine flow rate of a freshwater crayfish would be reduced when it is removed from water, as there is then no influx of excess water which must be removed. *Parastacoides tasmanicus* either has an extremely low urine flow rate in water, or else it exhibits a great reduction in flow rate when it is removed from water, because its combined urine, faeces and evaporative water losses at 99-100% R.H. and 15^{o}C were between 0.36% and 2.04% body weight per day. How *P. tasmanicus* disposes of its metabolic wastes under these conditions is unknown. It is possible that they are stored somewhere in the body, but no examinations were made to test this possibility.

Faecal water loss is also obviously low. The reduction in the rate of passage of food through the gut of dehydrating *P. tasmanicus* means that as the animals dehydrate, more water can be removed from the faeces. Even when the faeces are voided they might still be useful. Crayfish with their abdomens curled up, with the faeces retained within the fold, would have their pleopods and the ventral surface of the abdomen covered with faeces, which might reduce the rate of water loss from these areas.

The crayfish that died while attempting to moult suggests that *P. tasmanicus* is unable to moult out of water. This is not surprising, as during the late D stages, E and early A stages of the moult cycle the oxygen consumption of *P. tasmanicus* increases dramatically (see Chapter 6), and aerial respiration may be insufficient to meet the needs of a moulting animal , which could therefore die of asphyxiation. Also, without the lubricating effect and support of water, and the impossibility of absorbing water to increase turgor, the animals could find the physical act of moulting impossible. The cause of death of the moulting animal was not dehydration, as it had only lost 6% of its initial weight when it died.

Parastacoides tasmanicus does not have any specialised apparatus for taking up water directly from the substrate, such as is present in many terrestrial and semi-terrestrial crabs such as *Cardisoma, Gecarcinus, Ocypode, Sesarma* and *Uca* (Bliss, 1963, 1968; Bliss and Mantel, 1968; Gross, 1963; Harris, 1977; Wolcott, 1976). Wolcott (1976) described how *Ocypode quadrata* can take up water from soil containing as little as 3-5% water. This crab possesses tufts of setae at the bases of its second and third walking legs. These tufts are usually closely apposed and enclose the single posterior aperture to each branchial chamber. They are pushed into damp soil, and water moves amongst them and up them by capillary action. Water is 'sucked' out of the tufts into the gill chambers when the crab creates a partial vacuum in the gill chamber. The exact mechanism by which it achieves this has not been fully elucidated yet, but it obviously involves the scaphognathites. The water is taken up (from within the gill chambers) by sections of the gills.

Juvenile *P. tasmanicus* can apparently utilise water that is present in very small droplets, which larger animals are unable to use. This could be useful in burrows where the only available water is that which condenses on the walls of the burrow at night. Larger animals cannot use this source of water, but they could supply part of their requirements by eating a damp substrate such as mud or dew-covered leaves.

If access to water is denied, then small *P. tasmanicus* will lose water at a faster rate than larger animals, and will die sooner (see Figures 4.1a, 4.1b, 4.2a, 4.2b, and 4.4). This is the case with most other semi-terrestrial and terrestrial decapods such as *Cardisoma guanhumi* and *Ocypode quadrata* (Bliss, 1968; Henning, 1975; Herreid, 1969b) but not for *Gecarcinus lateralis* in which resistance to desiccation is independent of size (Bliss, 1968).

It is very difficult to find data with which to compare the survival time of *P. tasmanicus* at 15° and 20°C and 70%, 93% and 100% R.H. Most of the terrestrial and semi-terrestrial crabs are tropical, and because of this most tests on them have been carried out at temperatures in the vicinity of 30°C, and normally at lower R.H. than 70%. The comparable results that are available are summarised in Table 4.7.

The *Potamon* species referred to in Table 4.7 are all amphibious crabs, and are used to returning to water before they become dehydrated. The survival of *P. tasmanicus* in air appears to exceed that of these crabs, but their survival time was measured in moving air whilst that of *P. tasmanicus* was measured in still air, so the results are not strictly comparable. The only tests done on crayfish at a comparable temperature and R.H. (Bovbjerg, 1952) show that both the stream dwelling crayfish, *Orconectes propinquus*, and the pond dwelling crayfish, *Cambarus fodiens*, have a very poor survival capacity out of water. Even the terrestrial

crabs, with one exception, at their normal temperature and a moderate R.H., have a shorter survival time out of water than *P. tasmanicus*.

Table 4.7 Survival of decapod crustaceans at various relative humidities and temperatures.

	Temp. (°C)	R.H. (%)	Survival time* (h)	Source
Freshwater crayfish				
Parastacoides tasmanicus	15	70	248 ± 21 (6)	This study
" "	15	93	801 ±102 (6)	" "
" "	15	93	327 ± 23 (6)	" "
" "	15	100	799 ± 72 (6)	" "
" "	20	93	339 ± 48 (6)	" "
" "	20	93	336 ± 17 (6)	" "
Orconectes propinquus	20.5	66	6	Bovbjerg (1952)
Cambarus fodiens	20.5	66	12	" "
Freshwater amphibious crabs.				
Potamon depressus	20	90	95	Dandy & Ewer (1961)
" "	20	70	46	" " "
P. warreni	20	90	78	" " "
" "	20	70	36	" " "
P. sidneyi	20	90	160	" " "
" "	20	70	62	" " "
Terrestrial crabs				
Holthuisana transversa	25	0	150	**
Gecarcinus lateralis	30	78	89	Bliss (1968)
Cardisoma guanhumi	30	78	53	" "
Ocypode quadrata	30	78	20	" "

* Mean (± S.E. (N) for *P. tasmanicus* only)
** Calculated from data in Greenaway and MacMillen (1978) and MacMillen and Greenaway (1978), for a 10 g crab. It is probably a rough estimate only.

If allowance for the differences in normal environmental temperatures are made, then the survival of *P. tasmanicus* at various humidities compares favourably with that of most terrestrial crabs and far exceeds that of amphibious crabs, other freshwater crayfish and even amphipods (see Lagerspetz, 1963). It does not, however, compare favourably with the 'very terrestrial' *Holthuisana transversa* which can survive for years in a humid burrow without access to

surface water (MacMillen and Greenaway, 1978).

The relatively long survival time of *P. tasmanicus* out of water could be due to its having a higher L.W.L. or a lower R.W.L. than other species. Table 4.8 gives the lethal water loss of some brachyuran crabs, with which *P. tasmanicus* can be compared.

Table 4.8 Weight loss of brachyuran crabs dying from desiccation.

	Habitat	L.W.L.*	Source
Marine crabs			
Gecarcinus lateralis	Terrestrial	21	Bliss (1968)
Cardisoma guanhumi	Semi-terrestrial	16	" "
Ocypode quadrata	" "	14	" "
Uca minax	" "	18	Herreid (1969a)
Panopeus herbstii	Inter-tidal	25	" "
Menippe mercenaria	Aquatic	12	" "
Arenaeus cribrarius	Aquatic	14	" "
Freshwater crabs			
Holthuisana transversa	Terrestrial	31.2	Greenaway and MacMillen (1978)
Sudanonautes africanus africanus	Semi-terrestrial	20	Lutz (1969)
Potamon sidneyi	Semi-terrestrial	18	Dandy and Ewer (1961)
P. warreni	" "	15	" " "
P. depressus	" "	15.5	" " "

* (% initial body weight)

In general it appears that the more terrestrial crabs have a higher L.W.L. than less terrestrial species. The anomuran crabs have a higher L.W.L. than the brachyuran crabs; for example the semi-terrestrial anomuran, *Clibanarius vittatus* has a L.W.L. of 35% body weight (Herreid, 1969a).

Parastacoides tasmanicus was found to be able to tolerate a weight loss of approximately 16.5 - 26.5% before death depending on the conditions of the test, and this compares favourably with other decapods, even with many of the terrestrial species, in respect of the water loss that can be tolerated. Like other crustaceans, though, it does not

fare well when it is compared to other arthropods. Most terrestrial insects have a L.W.L. in the range of 30 - 60% body weight. For example, *Blatta orientalis*, *Rhodnius prolixus* and *Papillio japonica* have L.W.L. values of 42%, more than 40%, and 56% body weight respectively (Dandy and Ewer, 1961).

Although the L.W.L. is independent of temperature (within normal limits) and humidity for many animals, the R.W.L. is very dependent on both, i.e. increases in temperature and/or decreases in relative humidity usually increase R.W.L., so that comparisons between *P. tasmanicus* and other species for this parameter are harder to find. Table 4.9 shows the R.W.L. of some decapod species under specified conditions.

Table 4.9 R.W.L. of some decapods at certain temperatures and relative humidities.

	Temp. (°C)	R.H. (%)	R.W.L. (%/h)	Source
Marine crabs				
Gecarcinus lateralis	30	78	0.23	Bliss (1968)
Cardisoma guanhumi	30	78	0.32	" "
Ocypode quadrata	30	78	0.74	" "
Freshwater crabs				
Potamom depressus, *P. warreni*, *P. sidneyi*	20	70	0.30-0.45	Dandy & Ewer (1961)
	20	90	0.12-0.14	" " "
Freshwater crayfish				
P. tasmanicus†	15	70	0.078±0.005	This study
" "	15	93*	0.030±0.006	" "
" "	20	93*	0.102±0.037	" "
" "	15	93#	0.052±0.002	" "
" "	20	93#	0.052±0.001	" "
" "	15	100	0.034±0.011	" "

† Mean ± S.E. for 6 measurements
* 15°C-acclimated
\# 5°C-acclimated

As was noted for the L.W.L., when normal environmental temperatures are taken into consideration, *P. tasmanicus* compares favourably with other decapods with respect to the R.W.L. Similarly,when a comparison is made with some of the other arthropods such as the desert scorpion, *Hadrurus arizonensis*, which at 30°C and in dry air loses only 0.028% body weight per hour (Hadley, 1970), the water loss of all of the Crustacea seems excessive.

To summarize, adult *P. tasmanicus* have a high L.W.L. and a low R.W.L. when compared to other decapod crustaceans.

Crayfish with the integument covered with petroleum jelly lose water at approximately the same rate as untreated crayfish, indicating that the site of most water loss is the gill chambers. In support of this conclusion are other unreported experiments which suggest that a covering of shellac or some other waterproof coating on the integument of dead crayfish does not change their rate of water loss. Dead crayfish lose water at the same rate as living ones, and this might at first appear to contradict the above conclusions. However, *P. tasmanicus* does not irrigate its gills for more than a brief period following exposure to air, so it is not really surprising that live and dead crayfish should lose the same amount of water from the gill chambers, and hence their R.W.L. should be the same.

There are conflicting views on the major sites of water loss of the various crabs that have been studied, and it may be that different crabs do have major differences in their sites of water loss. The shore crab, *Carcinus maenas*, and the subtidal crab, *Portunus marmoreus*, lose water at the same rate, whether their integument is coated with silicone or not (Ahsanullah and Newell, 1977). However, the water loss of *Potamon* species decreases by 62% when the carapace is coated

with petroleum jelly. The water loss from *Potamon depressus* is distributed as 41% from the integument, 29% from the branchial chambers and 17% from the tail area (Dandy and Ewer, 1961) (it was not specified what constituted the integument). Herreid (1969b) concluded that most of the evaporative water loss of crabs from all habitats was across the integument. Unfortunately there are no comparative data from any of the freshwater crayfish.

The graphs relating water loss of *P. tasmanicus* to time after the commencement of dehydrating conditions are mostly almost straight lines (see Figures 4.1a, 4.1b, 4.2a, 4.2b, 4.3a, 4.3b). However, when animals are exposed to dehydrating conditions for prolonged periods, it is apparent that the rate of water loss at the end of the exposure period is slightly lower than the R.W.L. at the beginning of the experiment. Dandy and Ewer (1961) suggested that the permeability of the exoskeleton might decrease as it dried, and it is possible that this also occurs with *P. tasmanicus*. *Parastacoides tasmanicus* has a low rate of water loss when removed from water, and it is possible that this 'low permeability' to water could also reduce water uptake when the crayfish is in water. If this is the case then *P. tasmanicus* might not have to produce as much urine as other crayfish. This reduction in urine volume in an animal with low integumentary permeability could lead to a considerable reduction in ion losses, which would be a real advantage in a habitat with very low concentrations of many ions. Low permeability to water is likely to have arisen as a means of preventing ion loss rather than as a means of surviving out of water, although it is useful in both roles.

5. DIGESTIVE ENZYMES AND ASSIMILATION EFFICIENCY OF *Parastacoides tasmanicus*

5.1 Introduction

The gastric mills of freshly caught specimens of *Parastacoides tasmanicus* have been found to contain both plant and animal remains, with plant material making up the bulk of the contents. Most of the plant material consists of pieces of roots, while earthworms account for most of the animal remains (Lake and Newcombe, 1975). Specimens of *P. tasmanicus* kept in the laboratory will rapidly devour earthworms, and can catch and eat tadpoles (Newcombe, 1970 and personal observations), but they can survive for extended periods with no food other than button grass mud. This mud has a high organic content due to the large amounts of decomposing and living plant material that it contains, and *P. tasmanicus* will consume large quantities of it if no other food is available. It would appear that *P. tasmanicus* is a herbivore/detritivore for most of the time, eating plants in various stages of decomposition, but it is carnivorous when animal food is available. R. Swain (unpublished data) has studied the gastric mill structure of *P. tasmanicus* and has concluded, using the reasoning of Caine (1975), that the structure is that of a mainly herbivorous animal with some carnivorous traits.

Although the food of *P. tasmanicus* is known, it is not known how efficiently *P. tasmanicus* utilises this food. One approach to this problem is to study the type and activity of the digestive enzymes of the animal, and another approach is to study the assimilation efficiency of *P. tasmanicus* specimens maintained on controlled diets.

Yonge (1937) suggested that, in general, carnivores have strong digestive proteases and weak carbohydrases, while herbivores tend

to have more active carbohydrases and weaker proteases. Omnivores would be expected to have proteases and carbohydrases with activities between those of the herbivores and carnivores. It has been found, however, that amongst the invertebrates the distribution and activity of digestive enzymes such as proteolytic enzymes (Kozlovskaya and Vaskovsky, 1970), cellulases (Monk, 1976; Yokoe and Yasumasu, 1964) and other carbohydrases (Bjarnov, 1972; Nielsen, 1962) are not correlated with the type of food that animals eat, nor with their mode of life, but rather with their systematic position. Degkwitz (1957) studied the proteolytic enzymes of many crustaceans and concluded that in this group there was no relationship between the nature of the digestive enzymes and the modes of life of the investigated species. Sather (1969) disagreed with this conclusion to some extent, although he did find that three omnivorous decapods he tested had greater digestive amylase and proteinase activities than did carnivorous species. It will be useful to briefly review what is known about the more important digestive enzymes that have usually been found to be present in crustaceans, especially decapods, so that some idea can be obtained of what digestive enzymes *P. tasmanicus* might be expected to have. Of course the activities of the enzymes vary markedly from one species to another.

Fat-hydrolysing enzymes are apparently present in all crustaceans (Barker and Gibson, 1977; Barnard, 1973; Brockerhoff, Hoyle and Hwang, 1970; Brockerhoff, Stewart and Tacreiter, 1967; Kruger and Graetz, 1928; van Weel, 1960, 1970; von Buddenbrock, 1956; Vonk, 1960). Although within the decapods these enzymes usually seem to be esterases rather than lipases (von Buddenbrock, 1956; Vonk, 1960), some decapods, such as *Homarus* and *Panulirus*, digest fats better than esters of lower alcohols and lower fatty acids.

Crustacea, Asteroidea and certain species of Polychaeta, Sipunculidea, Loricata and Cephalopoda were found to have the highest proteolytic activity of 50 species of marine invertebrates tested by Kozlovskaya and Vaskovsky (1970). In fact, proteases have been found in all isopod, amphipod and decapod crustaceans tested (Agrawal,1963; Bernice, 1971; Bond, 1934; Brockerhoff *et al*, 1970; Brun and Wojtowicz, 1976; DeVillez, 1965, 1975; DeVillez and Buschlen, 1967; Hasler, 1935; Kruger and Graetz, 1928; Sather, 1969; van Weel, 1960), although their activity varies enormously. The protease activity in the freshwater crayfish, *Orconectes virilis*, was found to come mostly from one component of the digestive juice, although a number of other components were found to make minor contributions to the observed activity (DeVillez, 1965, 1975).

Amylases have been reported from all the decapod Crustacea, but as with the other enzymes mentioned so far, their activity varies considerably from species to species (Bond, 1934; Brockerhoff *et al*, 1970; Brun and Wojtowicz, 1976; Hasler, 1935; Kooiman, 1964; Sather, 1969; van Weel, 1960). Cellobiase and cellulase*, two carbohydrases which are (potentially) very important to herbivores and detritivores, occur sporadically in the invertebrates. Elyakova (1972) measured cellulase activity in 37 species of marine invertebrates and found that it was highest in some of the molluscs, especially the gastropods, but it was also present in the Crustacea that were tested. It has been reported a number of times in some, but not all, amphipods, (Agrawal, 1961; Monk, 1976, 1977; Wildish and Poole, 1970 (native cellulase)) and isopods (Hassall and Jennings, 1975 (native cellulase)). Among the decapods, cellulase has been found in the lobster, *Homarus gammarus* (Kooiman, 1964), and in the freshwater crayfish, *Astacus astacus* and *Procambarus clarkii* (Kooiman, 1964;

*See section 5.1.1 for definition

Yokoe, 1960) and there is some evidence that it occurs in *P. tasmanicus* (Newcombe, 1970). These cellulases are often produced both by the animal concerned and by bacteria in its digestive tract.

Cellobiase is found in isopods (Nielsen, 1962) and has also been detected in *Homarus gammarus* and *Astacus astacus* (Kooiman, 1964). This enzyme can be used by detrivores to break down cellobiose, a product of the action of microorganisms on the cellulose in detritus (Barlocher and Kendrick, 1975).

Chitinase, which would be of great use to carnivores that consume animals with chitinous exoskeletons, has been reported from *Homarus gammarus*, *H. americanus* and *Astacus astacus*, but the activity is low (Brockerhoff *et al*, 1970; Kooiman, 1964).

Even when a digestive enzyme is present, and its activity is high under normal conditions, it is not necessarily of benefit to the animal concerned. For example, gut extracts of *Gammarus pulex* hydrolyse native cellulose and other cellulose substrates *in vitro*, and it might therefore be expected that *G. pulex* could degrade plant cell wall material. However, it cannot, as it lacks the other enzymes needed to do so (Monk, 1977). The cell wall of plants is a very complex structure (see Dean (1976, 1978) for a description of the structure of the secondary layer of the cell wall, the part of the cell wall where most of the cell mass of wood is), and apart from cellulase other enzymes, including xylanase, mannanase and β-1,3-glucanase are needed to degrade it. The cellulase of *G. pulex* is probably restricted to digestion of the many small non-cellular particles, with exposed areas of cellulose which enter the gut. Similarly, the decapods, *Astacus astacus* and *Homarus gammarus*, both possess a cellulase, but only *A. astacus* has the enzymes necessary to allow it to digest the plant cell wall (Kooiman, 1964).

It is obvious that it is necessary to either test for a very large number of enzymes, or to back up an investigation of the activity of the major

digestive enzymes with an investigation of the efficiency with which a number of foods can be assimilated by an animal, if accurate information about the food sources useful to the animal concerned is to be obtained.

The assimilation efficiencies of different invertebrates feeding on various foods varies enormously (Breteler, 1975a; Buhr, 1976; Forster and Gabbott, 1971; Hågvar, 1975; Johannes and Satomi, 1967; Klekowski and Fischer, 1975; Kofoed, 1975; Richardson, 1975a, 1975b). In general, carnivores feeding on animal food have assimilation efficiencies which are very high, often approaching or exceeding 90% (Abolmasova, 1970; Conover and Lalli, 1974; Heiman and Knight, 1975; Mason, 1970), while animals feeding on plant and algal food usually have lower assimilation efficiencies, in the region of 30-75%, although higher values (up to 98% (Solatova *et al* 1969a, 1969b)) as well as extremely low ones have been recorded (Carefoot, 1973; Hågvar, 1975; Heiman and Knight, 1975; Klekowski and Fisher, 1975; Mason, 1970; Richardson, 1975a; Richman, 1958). Detritivores usually have the lowest assimilation efficiencies of any animals, with efficiencies sometimes as low as 5%, although efficiencies up to 50% also occur occasionally (Hargrave, 1970b; Mason, 1970; Richardson, 1975a; Yingst, 1976).

The presence and activity of the major digestive enzymes of *P. tasmanicus* and the assimilation efficiencies of the crayfish when eating a variety of foods, were determined, in order to reach conclusions about the ability of the crayfish to utilise the foods that are normally available to it.

5.1.1 Cellulases

Cellulases are defined as being 1,4-(1,3;1,4)-β-D-Glucan 4-glucanohydrolases (Enzyme Commission number 3.2.1.4 (Commission on Biochemical Nomenclature, 1973)) but are commonly called β-1,4-glucanases. These cellulases can be assayed with the aid of a number of substrates, the most commonly used ones being carboxymethylcelluloses (CMC), which are substituted, non-crystalline and fully water soluble β-1,4-glucans (Eriksson, 1969). However, many cellulases which will hydrolyse CMC will not hydrolyse 'native cellulose'. This led Reese, Siu and Levinson (1950) to postulate that 'real' cellulases contain a 'C_1' factor which converts native cellulose into a form that can be attacked by the 'C_x' factor (β-1,4-glucanase). They considered that the C_1-C_x complex was the real cellulase. They wrote: "The ability to develop an enzyme capable of hydrolysing the 1,4-β-glucosidic linkage found in cellulose and its derivatives is widespread among microorganisms. The ability of microorganisms to use native cellulose as a substrate is more restricted." This subject is treated in greater detail by Whitaker (1971), Norkrans (1967) and Furia, Gianfreda and Scardi (1975). For the purposes of this thesis cellulases detected with CMC as the substrate are referred to as being 'cellulases', while cellulases detected with cellulose as the substrate are referred to as 'native cellulases'. Where an animal is referred to as having a cellulase it does not necessarily mean that the animal does not have a native cellulase. It usually means that the presence or absence of cellulases has only been tested for using CMC as the substrate.

5.2 Materials and methods

5.2.1 Enzyme activity

Crayfish in the B and C stage of the moult cycle were killed by the shock of immersion in hot (80°C) water for a few seconds, after which they were rapidly cooled in cold water. This was the quickest method of killing the animals, and the short immersion could not have increased the temperature of the tissues of the digestive organs to the extent that the digestive enzymes were adversely affected. The midgut gland (the terms 'digestive gland' and 'hepatopancreas' are misnomers for this organ (van Weel, 1974)) was dissected out of each crayfish, weighed and then homogenized in 2-3 mL of ice-cold 5.4 M glycerol in a 10 mL Potter Elvehjem teflon tissue grinder. The homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was used as the midgut gland enzyme preparation. A drop of toluene was added to the enzyme extract to inhibit the growth of microorganisms. It was realised that the enzymatic activity in the macerated midgut gland was likely to be different from that in the tubules of the intact organ, but no other method of testing enzymatic activity was available, and this method still enabled presence or absence of enzymes, and their optimal pH, to be measured.

The stomach was dissected out intact, weighed and the contents homogenized in 2-3 mL of ice-cold 5.4 M glycerol. The empty stomach was reweighed and the weight of the gut contents was determined from the difference in the two weights. The homogenate was centrifuged and the supernatant was used as the stomach enzyme preparation. A drop of toluene was added to inhibit micro-organism growth. This method of extracting the enzyme solution did not allow the actual quantity of enzymes present to be measured, but it did enable measurements to be made of the activity of the enzymes in their diluted form as they occur naturally in the stomach.

The intestine was removed from each crayfish and the contents homogenized

in 1 mL of 5.4M glycerol, centrifuged and the supernatant used as the intestine enzyme preparation, where possible. In many cases there was insufficient material in the intestine to provide a useable quantity of 'concentrated' enzyme solution. For this reason the enzyme tests with intestine preparations were limited.

Crayfish were not starved before the tissues were removed for enzyme preparation. It was discovered very early in this investigation that enzyme activity was correlated with the fullness of the crayfish's digestive tract (see Table 5.1). Whether this was due to the stimulation of gastric secretion by the presence of food in the gut, as is known to occur in fish, e.g. *Carassius auratus* (Davies, 1964), and at least some decapod crustaceans (Barker and Gibson, 1977, 1978; Vonk, 1960), or whether the enzymes in the food itself were contributing to digestion, as found by Dabrowski and Glogowski (1977a, 1977b) for fish feeding on invertebrates, is irrelevant. The important question in this investigation was whether the enzymes were present or not.

Drops of digestive fluid from the different regions of the gut were placed on narrow range pH-indicator paper in attempts to measure the pH in the regions, but the digestive fluid affected the dyes in the paper so that no acceptable estimates were available.

5.2.1.1. Measurement of lipase and esterase activity

Lipase and esterase activity were determined by a 'continuous titration' technique (Bier, 1955; Brockerhoff *et al*, 1970; Desnuelle, 1961; Marchis-Mouren, Sarda and Desnuelle, 1959; Naher, 1974; Wills, 1954). The substrates used were:

(a) for lipase determination:
a mixture of 0.5 mL of {2mL triolein[1] homogenized in 5 mL of 10% gum arabic}[2]
0.5 mL of 10% bile salts[3]
1.0 mL of 0.1M $CaCl_2$
14.0 mL of distilled water

1. Triolein is a specific substrate for lipases (Naher, 1974).

2. Bile salts aid in the emulsification of the substrate (Fiore and Nord, 1949).
3. Calcium chloride helps to stabilise the enzyme, stimulates the rate of reaction and removes the fatty acids produced by enzyme hydrolysis of fats, as insoluble soaps (Balls, Matlack and Tucker, 1937; Wills, 1960).

(b) for esterase determination:

a mixture of 0.5 mL of {2 mL tributyrin[1] homogenized in 5 mL of 10% gum arabic}
15.5 mL of 0.1M $CaCl_2$

1. Tributyrin is usually considered to be a substrate for lipase, but the true substrates for lipase are long chain triglycerides. Lipase does attack tributyrin, but esterases - a poorly defined term - are likely to have a greater effect. In addition, some of the common substrates used to test for esterases, such as p-nitrophenyl acetate, are not specific, as they are also attacked by trypsin and chymotrypsin (Desnuelle, 1961).

The substrate mix, except for the triolein or tributyrin fraction, was placed in a 25 mL flat-bottomed flask. The flask was placed in a dish which contained water that was being continuously recycled through a constant temperature water bath. Except for the experiments that tested the effect of temperature on the enzyme activity, the temperature of the water in the dish was maintained at 37.0 ± 0.2°C. The dish was placed on top of a magnetic stirrer, and a small magnetic stirring bar was put in the flask to keep the substrate well mixed. The mouth of the flask was wide enough to allow entry of, (a) the end of a Pasteur pipette through which high-purity nitrogen (Commonwealth Industrial Gases) flowed; this prevented oxidation and CO_2 contamination of the contents of the flask, (b) the electrode of a Townson digital pH meter, (c) the tip of a 1 mL gold-line burette which was filled with CO_2-free 0.01M NaOH and (d) a thermometer. When the substrate had reached the temperature of the water bath the 0.5 mL of the triolein or tributyrin fraction was added to the flask, and the pH of the substrate mix was adjusted to the required pH by the addition of 0.1M NaOH through a gap in the top of the flask. The pH was kept constant for 8 minutes with additions of further quantities of 0.1M NaOH. For a further 4 minutes 0.01M NaOH was added from the 1 mL burette to keep the pH constant. The volume of 0.01M NaOH added

from the burette was noted and was designated V_1. This was a measure of the quantity of alkali that had to be added to neutralise the fatty acids produced by 'spontaneous' hydrolysis.

A measured quantity (0.1 - 0.3 mL) of enzyme extract was added to the flask, and the pH was adjusted to the required pH and maintained there for 1 minute, by addition of more 0.1M NaOH. For the next 4 minutes 0.01M NaOH was added from the 1 mL burette to maintain the required pH. This volume was designated V_2, and was a measure of the quantity of alkali that had to be added to neutralise the fatty acids produced by both 'spontaneous' hydrolysis and by the action of the enzymes.

As 1 mL of 0.01M NaOH is equivalent to the release of 10 μmol of fatty acids from the lipid substrate, then

$(V_2 - V_1) \times 10/4$ = μmoles of fatty acids released per volume of enzyme solution per minute.

The activity was converted to μmoles of fatty acids (FA) released per gram of tissue per minute. It seemed more appropriate to measure activity in terms of weight of tissue rather than in terms of protein content, as this method allows one to estimate the amount of substrate that could be digested in a given time by the stomach, midgut gland or intestine.

5.2.1.2. Measurement of protease activity

Protease activity was determined with casein as the substrate and spectrophotometric measurement of the amino acids released by the action of the enzyme (Kunitz, 1947). 0.25 g of 'Hammarsten' casein was suspended in 25 mL of buffer in 100 mL bottles. Initially a citrate-phosphate buffer was used for the pH range of 5 - 7, and a boric acid-phosphate buffer was used for pH above 7. (The buffers were made according to Gomori, 1955). For later experiments the 'Universal' buffer of Northrop (1922) was found to be more suitable and was used for the entire pH range of 4 - 10, which was the extent of the pH range tested. The casein suspensions were heated in a boiling-water bath for 15 minutes, which resulted in the dissolution of the casein, then cooled and stored at $4^{o}C$ until required. They were always used within 2 days

of being prepared.

A series of 15 mL centrifuge tubes, each containing 1 mL of buffered casein, were heated to 37 ± 0.2°C in a constant temperature bath (except in the experiments in which the effect of temperature on the activity of protease was determined) for 5 minutes. A measured quantity of enzyme solution (0.1 - 0.2 mL) was added to each of the centrifuge tubes, and the contents were mixed for a few seconds with a 'Super-mixer' vortex stirrer. The tubes were each stoppered with a glass marble and incubated for exactly 20 minutes. The reaction was stopped by the addition of 4 mL of 0.3M trichloroacetic acid (TCA) to each of the tubes. Blanks consisted of 1 mL of casein solution and 0.1 - 0.2 mL of buffer solution treated in an identical manner to the test samples, and controls consisted of 1 mL of casein solution to which was added 4 mL of 0.3M TCA before the enzyme solution was added. All of the tubes were left at room temperature for 1 hour and then centrifuged (3000 rpm for 10 minutes). The absorbance of the supernatant was measured at 260 and 280 nm in 1 cm pathlength silica-glass cells in a Cecil 292 spectrophotometer. The formula of Layne (1957) was used to determine the concentration of 'split products' (mainly amino acids and very short peptides), while taking into account any absorbance due to the presence of nucleic acids. After allowances were made for amino acids in the enzyme solution, the protease activity was calculated as mg split products released/g tissue.min^{-1}.

5.2.1.3. Measurement of cellulase and amylase activity

There are a number of methods available for measuring amylase and cellulase activities, and several of these were tried. The labelled-starch method for measuring amylase activity (Rinderknecht, Wilding and Haverback, 1967) and the substrate-film method of Sumner (1968), which determines the presence or absence of cellulase activity, were both tried but were found to be unsatisfactory. The viscosimetric method of determing cellulase activity (Almin and Eriksson, 1967, 1968; Almin, Eriksson and Jansson, 1967) is ideal for measuring β-1,4-glucanase activity on soluble substates such as

carboxymethylcellulose (CMC), but is unable to measure enzyme activity on insoluble substrates. The method found to be most suitable, and the one used here, was the measurement of cellulase activity by the 'reduction power' estimation on CMC or other cellulose substrate (Furia *et al*, 1975). This measures not only β-1,4-glucanase activity, but also β-glucosidase (cellobiase) activity. The β-1,4-glucanase depolymerises CMC (or cellulose) by splitting it into reducing fragments, which in turn are attacked by the β-glucosidase with the ultimate production of glucose (provided that at least one of the anhydroglucose units in the reducing fragment is unsubstituted). The total enzyme activity is measured by determining the amount of reducing sugars produced per unit time. In fact, both amylase and cellulase activities were measured by determining the reducing sugars produced (Rick and Stegbauer, 1974) by the action of enzymes on the appropriate substrates.

The substrate used in the amylase determination was 1% soluble starch in either phosphate-citrate buffer (pH 3.4 - 7.0) or phosphate buffer (pH 6.8 - 8.0) (Gomori, 1955). 1% solutions of CMC (Hercules type 7MF) in the appropriate buffers were used in the tests for cellulase activity. Other tests were made at pH 7.0 (phosphate buffer) for native cellulase activity, with substrates of cellulose powder (Whatman CC 41 microgranular cellulose (TLC) powder), filter paper (with the fats removed), and cotton wool (fats removed). 50 mg of insoluble substrate per 2 mL of buffer were used.

2 mL of substrate solution were incubated with 0.05 mL of enzyme solution in stoppered test tubes for 20 minutes at 25 ± 0.2°C. The insoluble substrates were incubated with the enzyme solution for 2½ hours. Blanks consisted of substrates without

enzyme solution. The reaction was stopped by the addition of 2 mL of dinitrosalicylic acid reagent (0.04M 3,5-dinitrosalicylic acid + 1.33M potassium tartrate + 0.38M sodium hydroxide in distilled water) to each test tube. 0.05 mL of enzyme solution was then added to the blanks. The test tubes were placed in a boiling-water bath for 5 minutes, cooled, centrifuged (3000 rpm for 10 minutes), and the absorbance of the supernatant measured at 546 nm in a Cecil 292 spectrophotometer after 20 minutes. Standards of maltose were used, and the results were converted to mg reducing sugars produced/g tissue.min^{-1}.

5.2.1.4 Determination of presence or absence of chitinase

No determination of the absolute activity of chitinase was made, but its presence, and the effect of pH on it, were established. The method used was based on the determination of N-acetyl-D-glucosamine released from chitin by chitinase (Jeuniaux, 1966; Reissig, Strominger and Leloir, 1955).

One mL of a suspension of powdered chitin (20 mg/mL) in water was added to mixtures of 2 mL of citric acid-phosphate buffer (pH 5.2 - 7.0), 0.2 mL of enzyme solution and 3 drops of toluene, in stoppered test tubes. 1 mL samples were taken immediately, and again after 24 hours incubation at 37.0 ± 0.2^{o}C. These samples were added to 1 mL distilled water in test tubes, and heated in a boiling-water bath for 10 minutes. They were then centrifuged, after which sub-samples of 0.5 mL of the supernatant were taken and added to 0.2 mL saturated sodium borate solution. The sub-samples were heated in a boiling-water bath for 5 minutes, cooled, and 3 mL of a p-dimethylaminobenzaldehyde (DMAB) solution (1 g DMAB in 1.25 mL of 10N HCl and 98.75 mL of glacial acetic acid, prepared just prior to use) added. The solutions were mixed and allowed to stand for 20 minutes at 37.0 ± 0.2^{o}C, after which they were cooled and the

absorbance at 585 nm measured in a Cecil 292 spectrophotometer.

The temperatures at which the activities of the various digestive enzymes were measured were considerably above normal environmental temperatures, in order to increase activities to levels where substantial activity could be measured in a reasonable time. In most cases the temperature was 37°C but for amylase and cellulase, measurements at 25°C proved to be adequate.

5.2.2 Assimilation efficiencies of *Parastacoides tasmanicus* on controlled diets

Crayfish were starved until the mid- and hind-gut, and presumably the stomach were empty. The mid- and hind-gut can be seen through the exoskeleton (with the aid of a strong light), and the presence or absence of material in them determined. Animals kept at 15°C were starved for about 2 days, while animals kept at 5°C had to be starved for 3 - 4 days. Once their guts were empty the crayfish were placed in clean containers with 200 mL of treated button grass water.

In early experiments the crayfish were fed a known weight of live, starved (so that their guts were empty) worms, which were usually eaten within 1 minute. The faeces produced by the crayfish were collected frequently and dried at 60°C in a vacuum oven. When no more faeces were produced the energy content of the accumulated dried faeces was measured in a micro-bomb calorimeter (Phillipson, 1964). The wet:dry weight ratio and energy content of dried worms were also determined.

For later experiments, when crayfish were fed a variety of plant and animal foods, a different method was used to collect faeces. The abdomen of each starved crayfish was placed in the end 2.5 - 5.0 cm of a condom, which was held in place at the cephalothorax-abdomen junction with a rubber band. So long as the rubber band was not too tight the crayfish was not harmed, while the condom formed a leakproof receptacle for the faeces. Crayfish showed no ill effects from this treatment, even when it was continued for several weeks.

The crayfish were fed on plant or animal food, which was left for 24 - 48 hours, after which time any uneaten food was removed and the amount eaten was determined. After the crayfish had been left long enough to allow the food to pass through the gut, the condom was removed from each one, and its contents washed out into aluminium pans. These faeces samples were dried in a vacuum oven at 60^{o}C and their energy content was measured. The wet : dry weight ratio of the food, and its energy content were also determined.

The assimilation efficiency was calculated and expressed as a percentage in terms of weight and/or energy i.e.

$$\frac{\text{dry weight of food - dry weight of faeces}}{\text{dry weight of food}} \times 100$$

or

$$\frac{\text{energy content of food - energy content of faeces}}{\text{energy content of food}} \times 100$$

The method used here includes the peritrophic membrane in the faeces weight, but this probably contributes less than 1% of the weight of the faeces (Lautenschlager, Kaushik and Robinson, 1978). The peritrophic membrane serves to keep the faeces neatly 'parcelled' so they can be easily collected, and to slow up the loss of the soluble parts of the faeces.

This method of estimating assimilation efficiency does not take into account the loss of organic material by any path other than via the anus.

5.3 Results

5.3.1. Enzyme activity

5.3.1.1 Lipase and esterase activity

The first three tests for lipase activity were carried out at pH 7.5 and 37.0 ± 0.2°C. These tests suggested that lipase activity of sections of the crayfish digestive system was affected by the amount of food in the stomach (see Table 5.1).

Table 5.1 Lipase and esterase activity in sections of the digestive system of *Parastacoides tasmanicus*

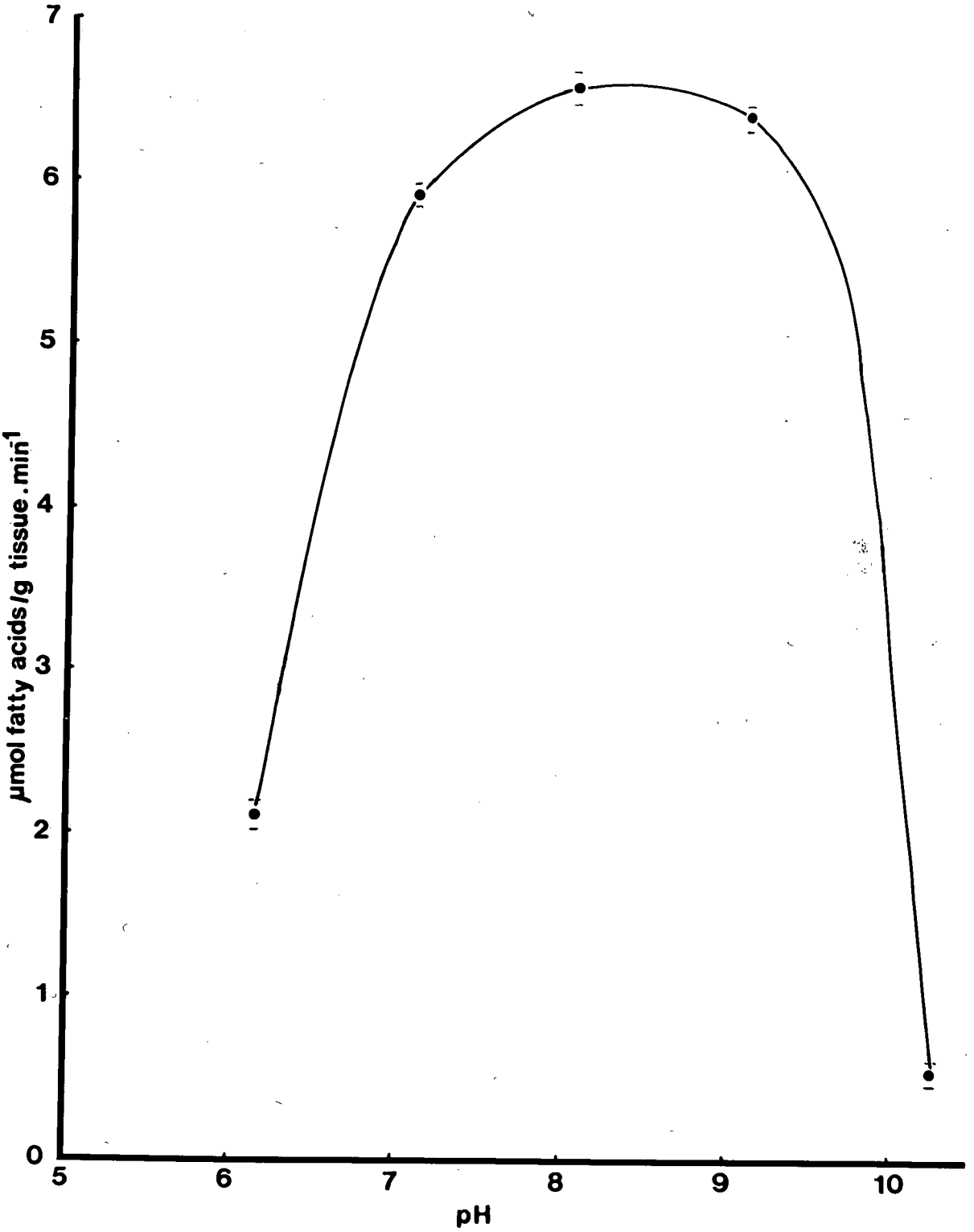
State of Stomach	Midgut gland Lipase activity	Stomach (μmol fatty acid/g	Intestine tissue.min^{-1})
Empty	12.8	1.8	-*
¼ Full	4.1	2.8	-*
Full	43.2	9.5	-*
	36.4		
	33.5		
	Esterase activity (μmol	fatty acid/g tissue.min^{-1})	
Full	250.0	15.7	37.1
	252.1	15.5	
	223.9		

*No activity detected

Although the relationship between the amount of food in the stomach and the lipase activity of the midgut gland was not as strong as the relationship between the amount of food in the stomach and the lipase activity in the stomach, it was strong enough to suggest that the lipase activity in the midgut gland was at least partly due to the presence of 'digestive' lipase (as distinct from lipase activity associated with functions of the midgut gland not related to digestion). As the midgut gland lipase extract is more active than the stomach extract, the lipase activity/pH curve shown in Figure 5.1 was derived using midgut gland lipase. The optimum

Figure 5.1 Effect of pH on lipase activity of midgut gland extract of *Parastacoides tasmanicus*, at 37.0 ± 0.2°C (Mean and range of three measurements).

Figure 5.1



pH of this enzyme appears to be in the range of pH 8.0 - 8.5.

The esterase activity of a crayfish with a full stomach is also shown in Table 5.1. The tests were carried out at pH 7.5 and at 37.0 ± 0.2^{o}C. Obviously there is esterase activity in all sections of the gut investigated; as with the lipase the highest activity occurred in the midgut gland, but in contrast appreciable activity was also found in the intestine. The pH/activity curve of esterase is shown in Figures 5.2a and 5.2b (extracts from two different animals). The optimum pH of esterase is in the range of pH 7.5 - 8.5.

The activity of esterase over a wide temperature range and at pH 8.0 is shown in Figure 5.3. The curve is similar to that of many other enzymes. Activity increases as the temperature increases until a temperature is reached at which the enzyme structure is adversely affected, and this results in a decreasing activity with further temperature increases.

5.3.1.2 Protease activity

The activity of protease in the midgut gland, stomach and intestine, and the effect of pH on this activity is shown in Figures 5.4a and 5.4.b. Once again the enzyme activity in the intestine is much lower than in the other sections of the digestive system that were tested. Figure 5.4b shows the effect of pH on the protease activity of the stomach and midgut gland over a wider range of pH than in Figure 5.4a, with one buffer being used over the entire range, rather than the two buffers that were used in the tests which produced Figure 5.4a. The changeover from one buffer to the other probably caused the 'bump' between pH 6.5 and 7.5 in Figure 5.4a. Despite these minor differences, the optimum pH in both series of tests was the same, namely pH 6.0, and for this enzyme the activity seems to be higher in the stomach than in the

Figure 5.2a and Figure 5.2b

Effect of pH on esterase activity of midgut gland extract of *Parastacoides tasmanicus*, at 37.0 ± 0.2 °C (extracts from two different animals).

Figure 5.2a

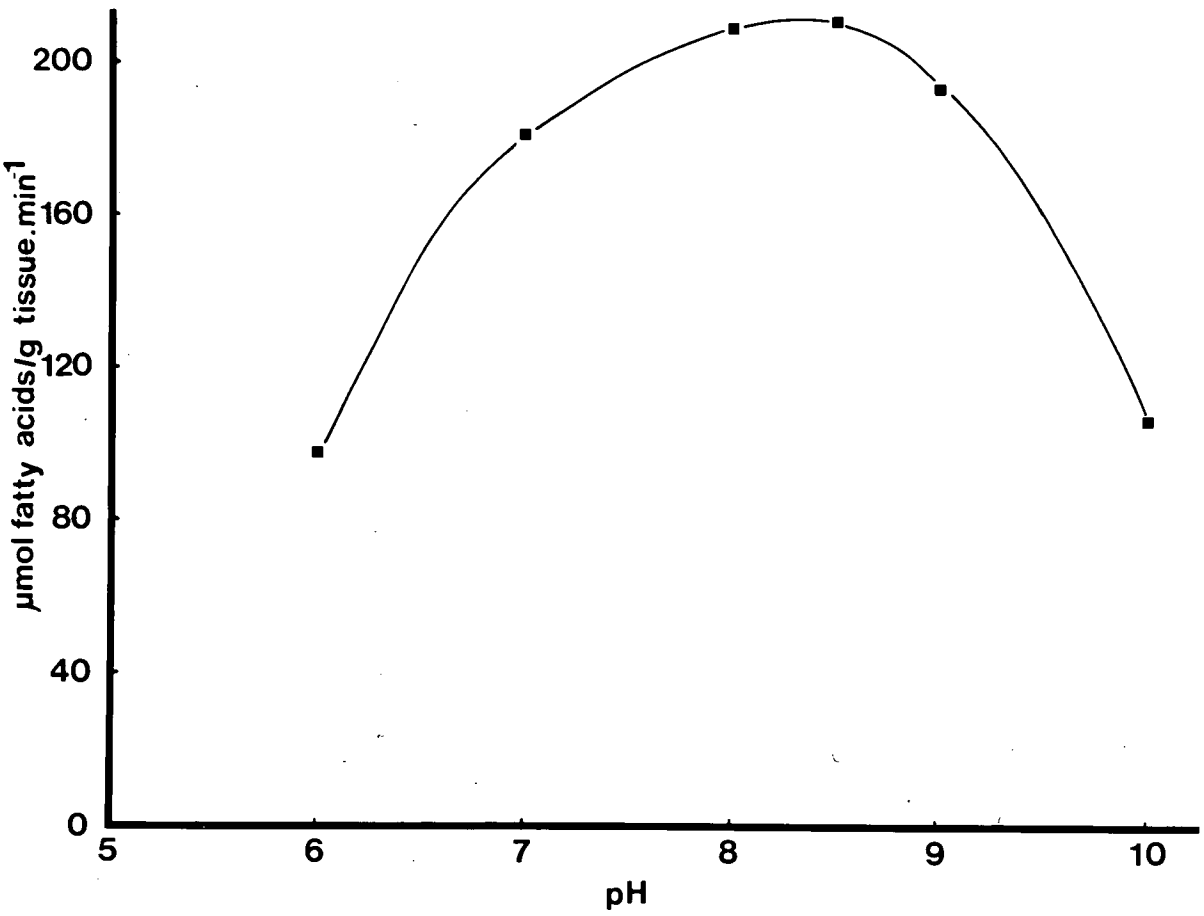


Figure 5.2b

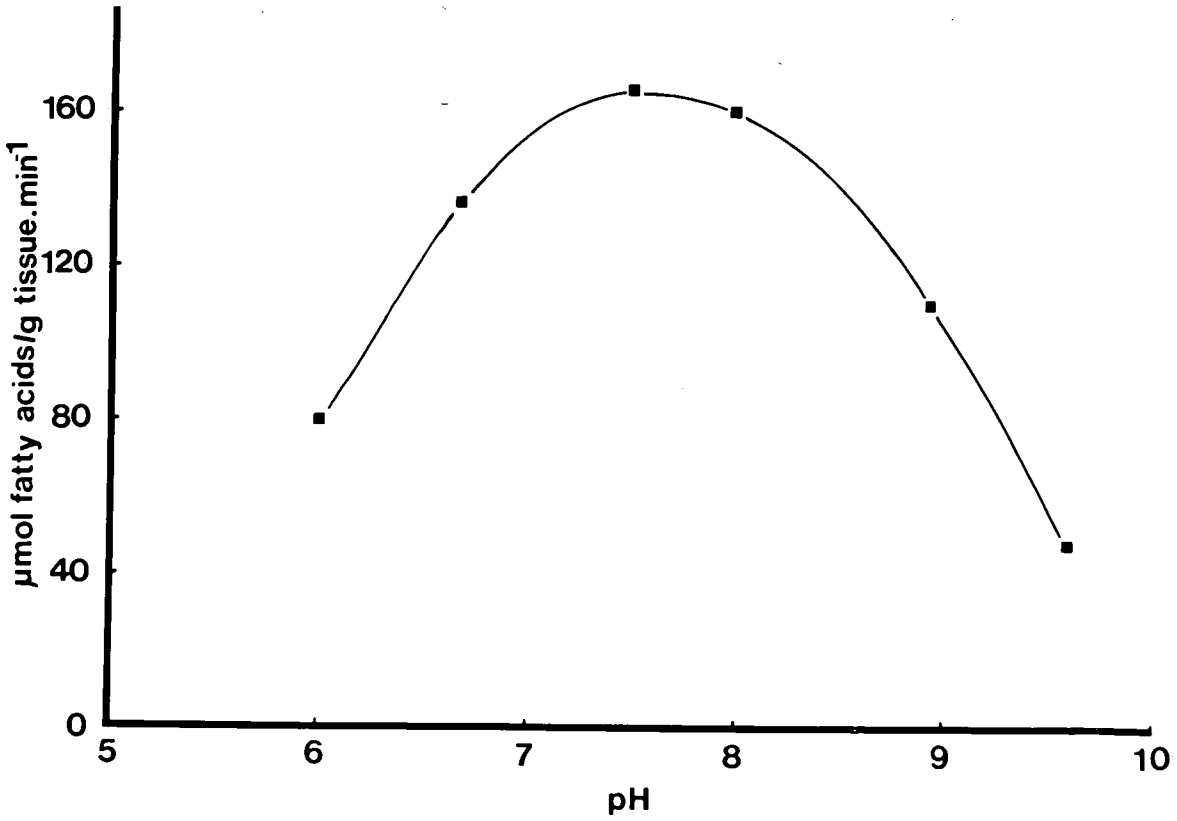


Figure 5.3 Effect of temperature on esterase activity of midgut gland extract of *Parastacoides tasmanicus*, at pH 8.0 (Mean and range of three measurements).

Figure 5.3

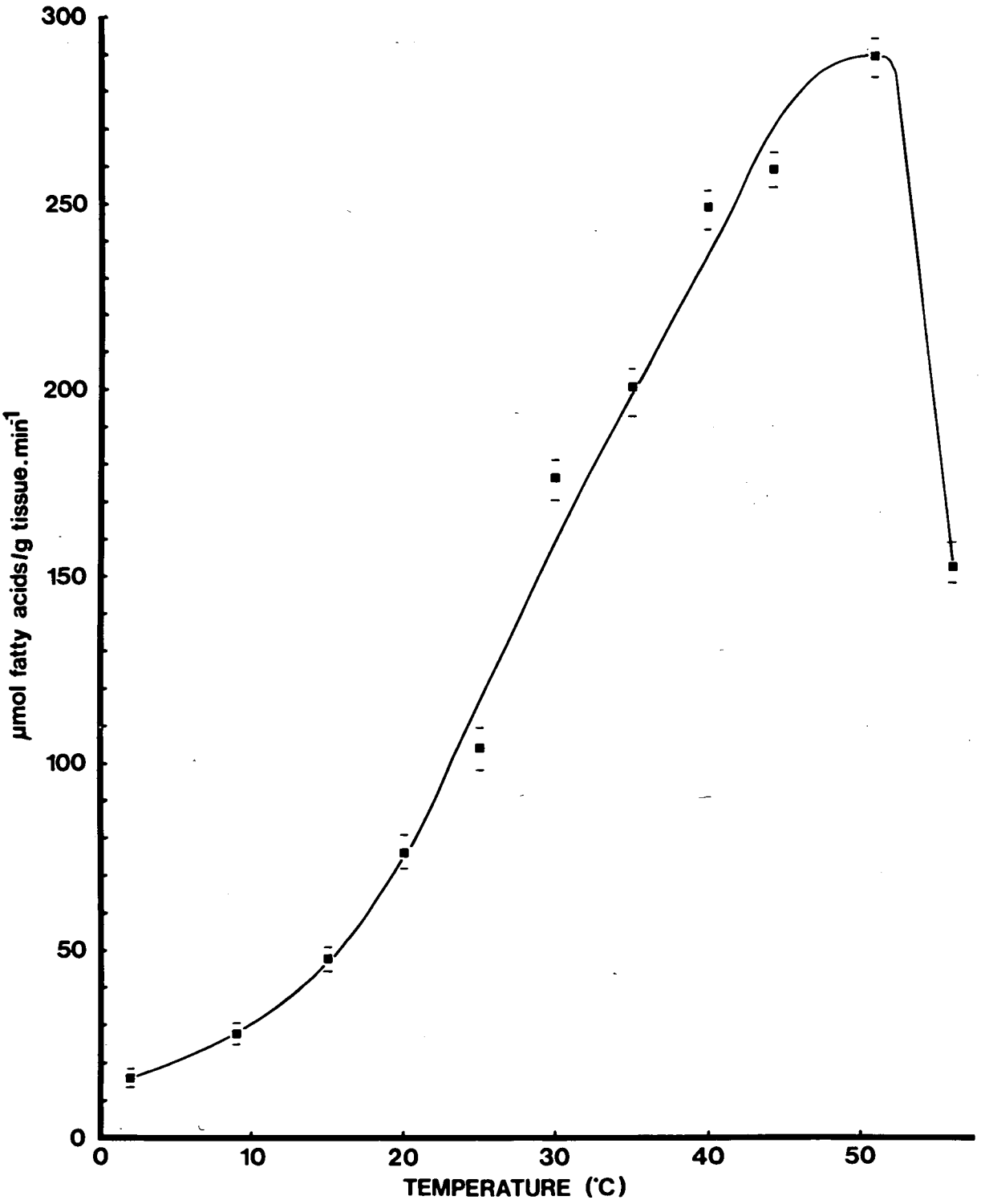

300
250
200
150
100
50
0
µmol fatty acids/g tissue.min-1
0
10
20
30
40
50
TEMPERATURE (°C)

Figure 5.4a Effect of pH on protease activity of midgut gland (■), stomach (●) and intestinal (▲) extracts of *Parastacoides tasmanicus*, at 37.0 ± 0.2°C. A citrate-phosphate buffer was used for pH 5 - 7 and a boric acid-phosphate buffer was used for pH above 7. (Mean and range of three measurements).

Figure 5.4b Effect of pH on protease activity of midgut gland (■) and stomach (●) extracts of *Parastacoides tasmanicus*, at 37.0 ± 0.2°C. The 'Universal' buffer of Northrop (1922) was used for the entire range of pH tested, (Mean and range of three measurements).

Figure 5.4a

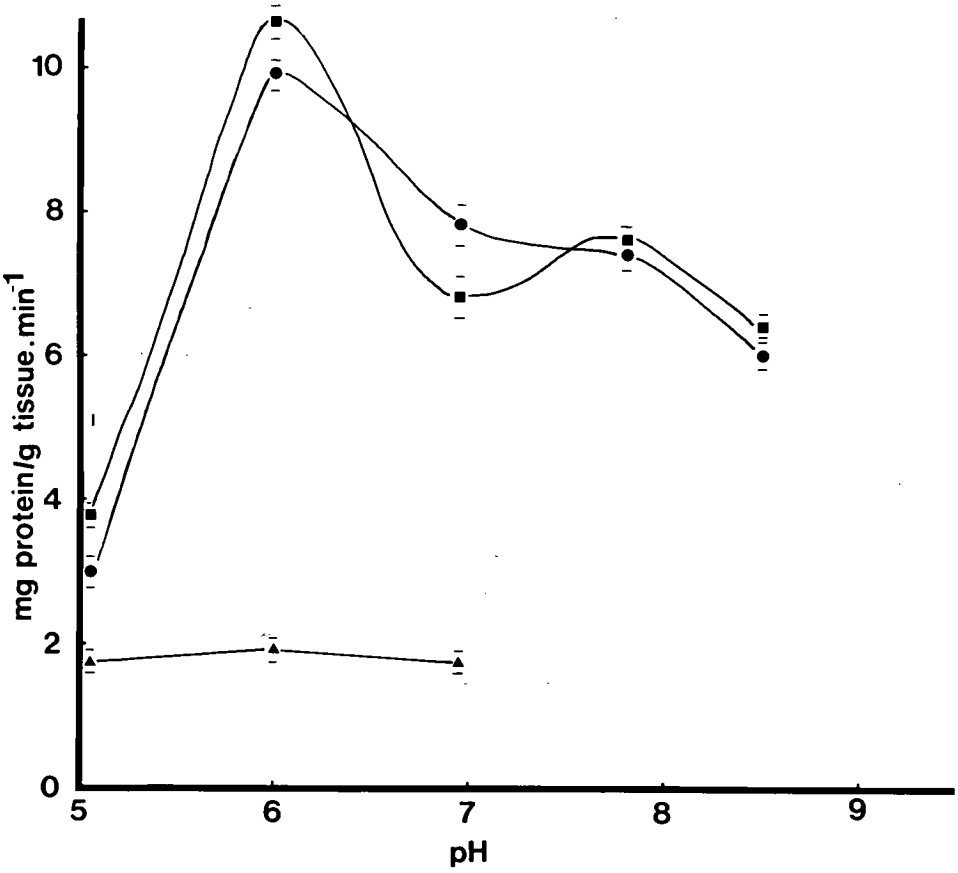
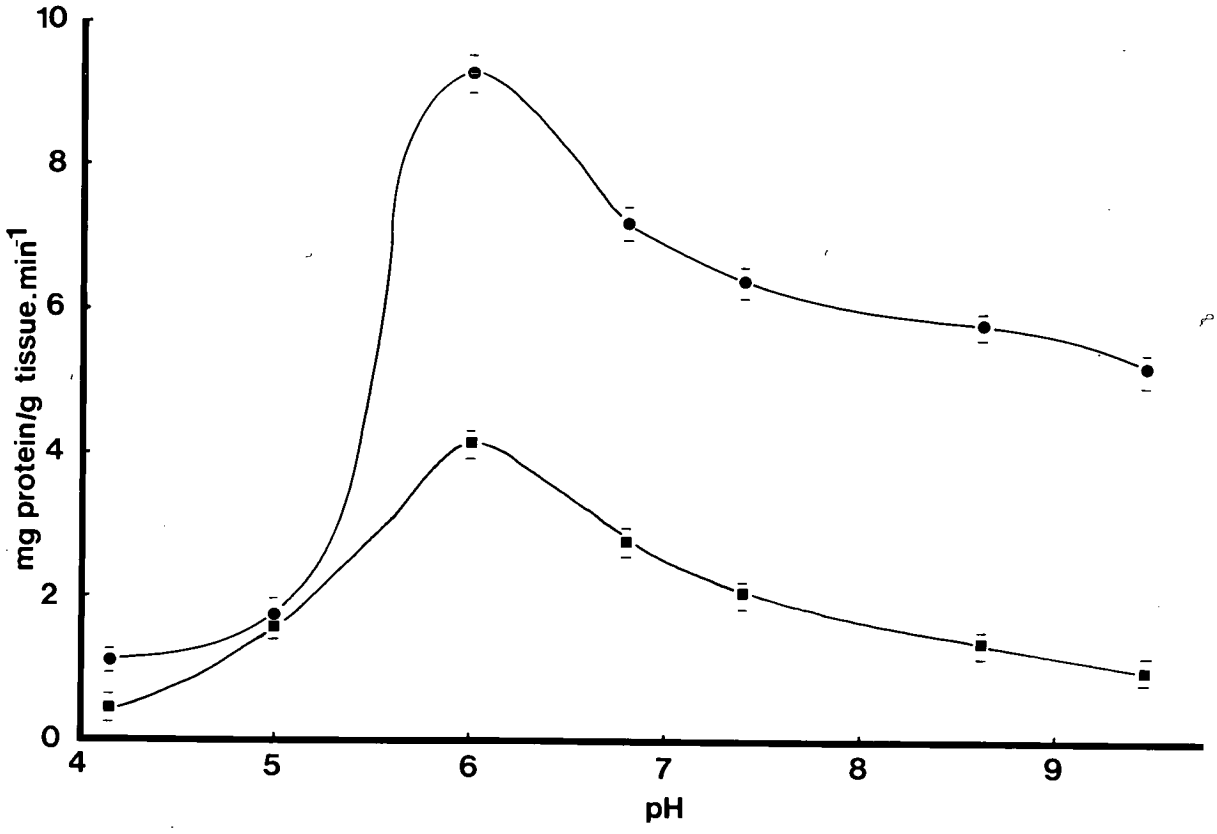


Figure 5.4b



midgut gland, with the intestine having a much lower activity than the other two sites.

The effect of temperature on protease activity at pH 6.0, as shown in Figure 5.5 is, not unexpectedly, very similar to the effect of temperature on esterase activity. Both the stomach and midgut gland extracts have maximum activity at a temperature of approximately 45^{o}C.

5.3.1.3 Cellulase and amylase activity

Amylase activity, as shown in Figure 5.6 has an optimum pH of 6.3, with the activity in the stomach exceeding that in the midgut gland. The amylase of *P. tasmanicus* has a narrower range of pH over which it is active than any of the other enzymes investigated, except chitinase.

Cellulase activity has an optimum pH of 7.0, and the activity in the stomach is higher than that in the midgut gland (see Figure 5.7). The maximum activity of the cellulase is approximately 16 mg reducing sugars produced/g tissue.min^{-1} for the stomach extract, and over 12 mg reducing sugar produced/g tissue.min^{-1} for the midgut gland extract. The native cellulase activity is much lower. Midgut gland extracts from the crayfish tested had an activity of 0.72, 0.51 and 0.0 mg reducing sugar produced/g tissue.min^{-1} when acting on cellulose powder, filter paper and cotton wool respectively, at pH 7.0.

5.3.1.4 Chitinase

Chitinase was found to be present in both the midgut gland and the stomach of *P. tasmanicus*. The chitinase has its maximum activity at pH 6.5, and the activity is reduced markedly if the pH departs even slightly from this (see Figure 5.8). The activity of this enzyme is higher in the stomach than in the midgut gland.

Figure 5.5 Effect of temperature on protease activity of midgut gland (■) and stomach (●) extracts of *Parastacoides tasmanicus*, at pH 6.0, (Mean and range of three measurements).

Figure 5.5

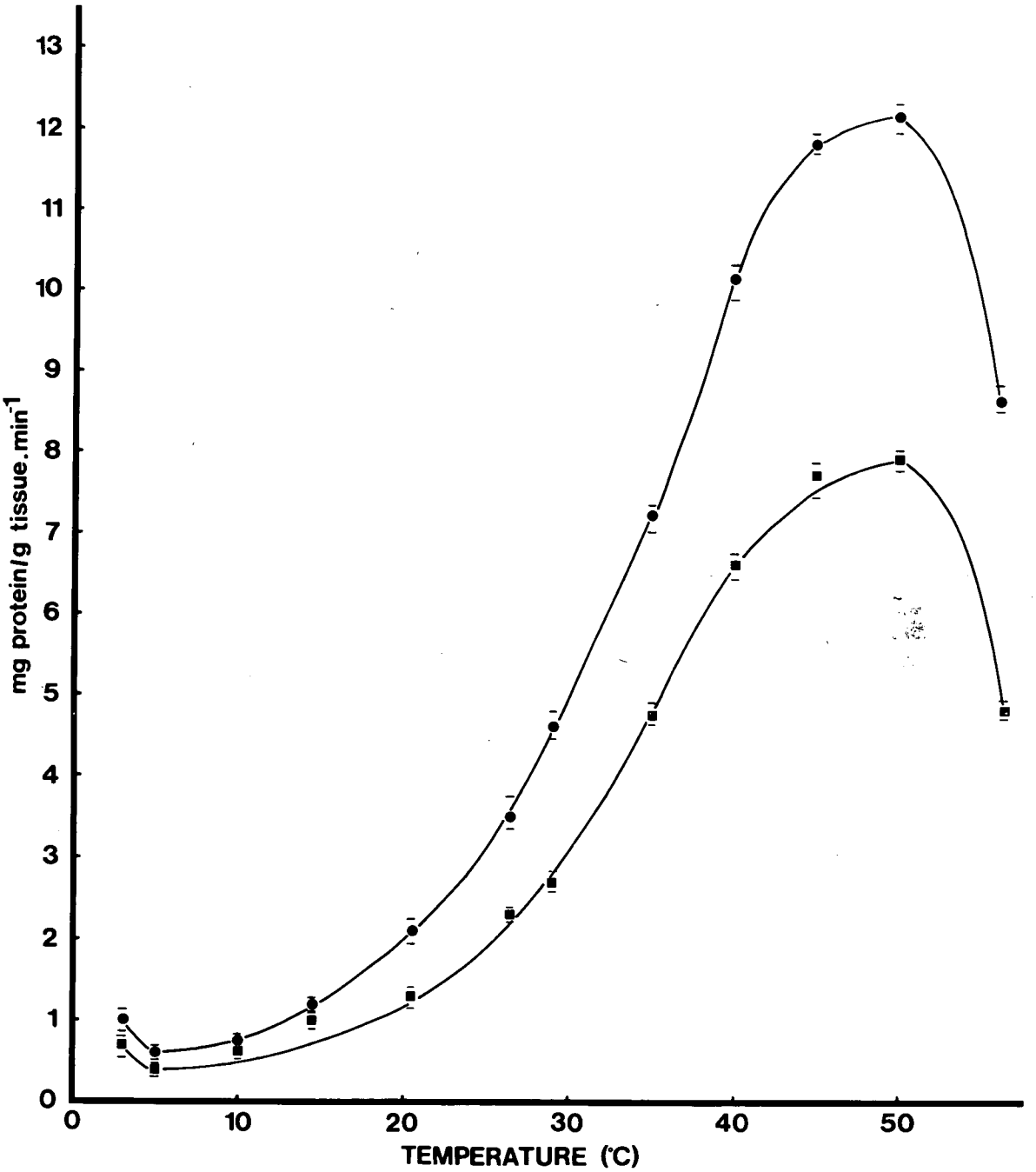

13
12
11
10
9
8
7
6
5
4
3
2
1
0
mg protein/g tissue.min-1
0
10
20
30
40
50
TEMPERATURE (°C)

Figure 5.6 Effect of pH on amylase activity of midgut gland (■) and stomach (●) extracts of *Parastacoides tasmanicus*, at 25.0 ± 0.2°C, (Mean and range of three measurements).

Figure 5.6

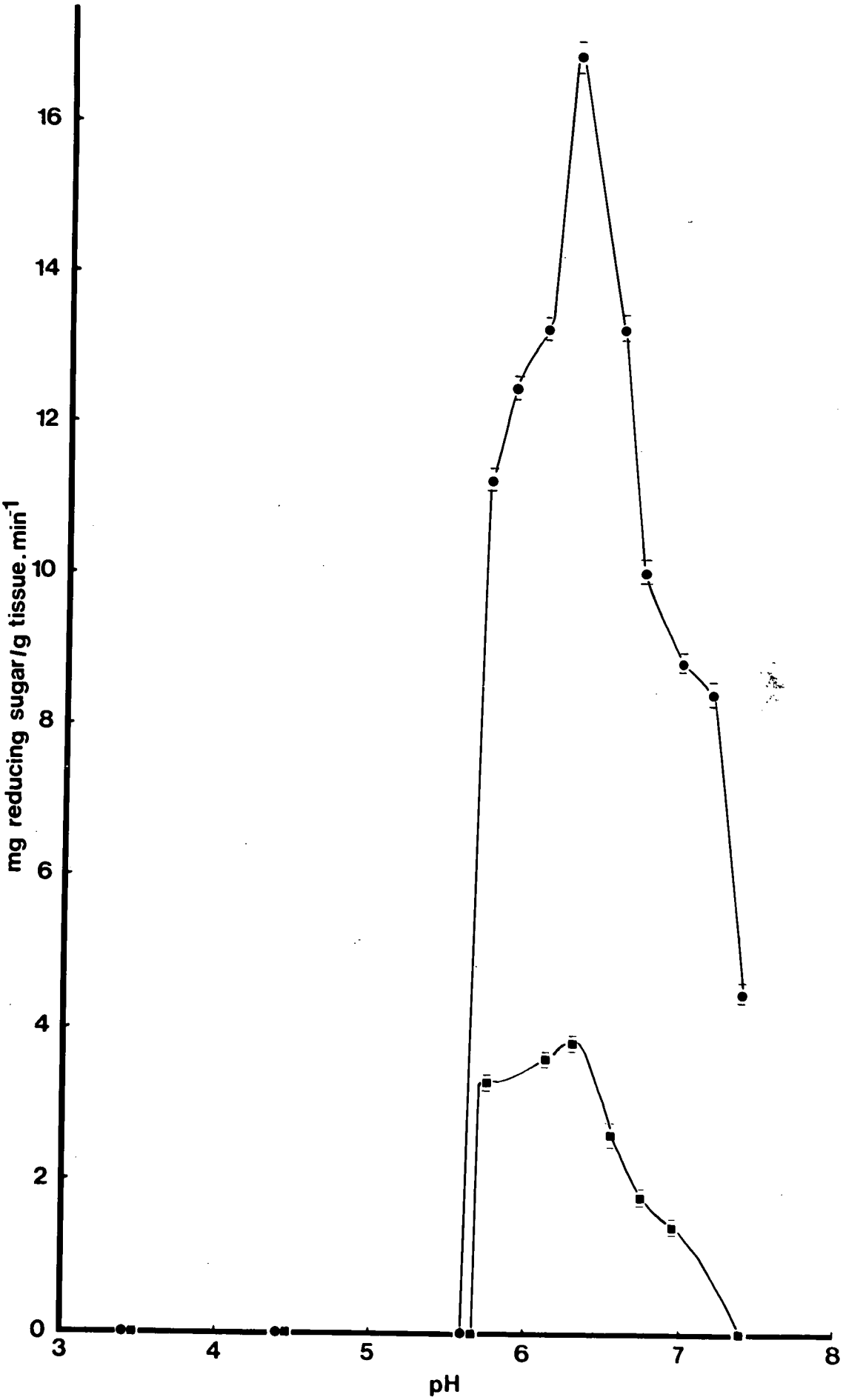


Figure 5.7 Effect of pH on cellulase activity of midgut gland (■) and stomach (●) extracts of *Parastacoides tasmanicus*, at 25.0 ± 0.2°C, (Mean and range of three measurements).

Figure 5.7

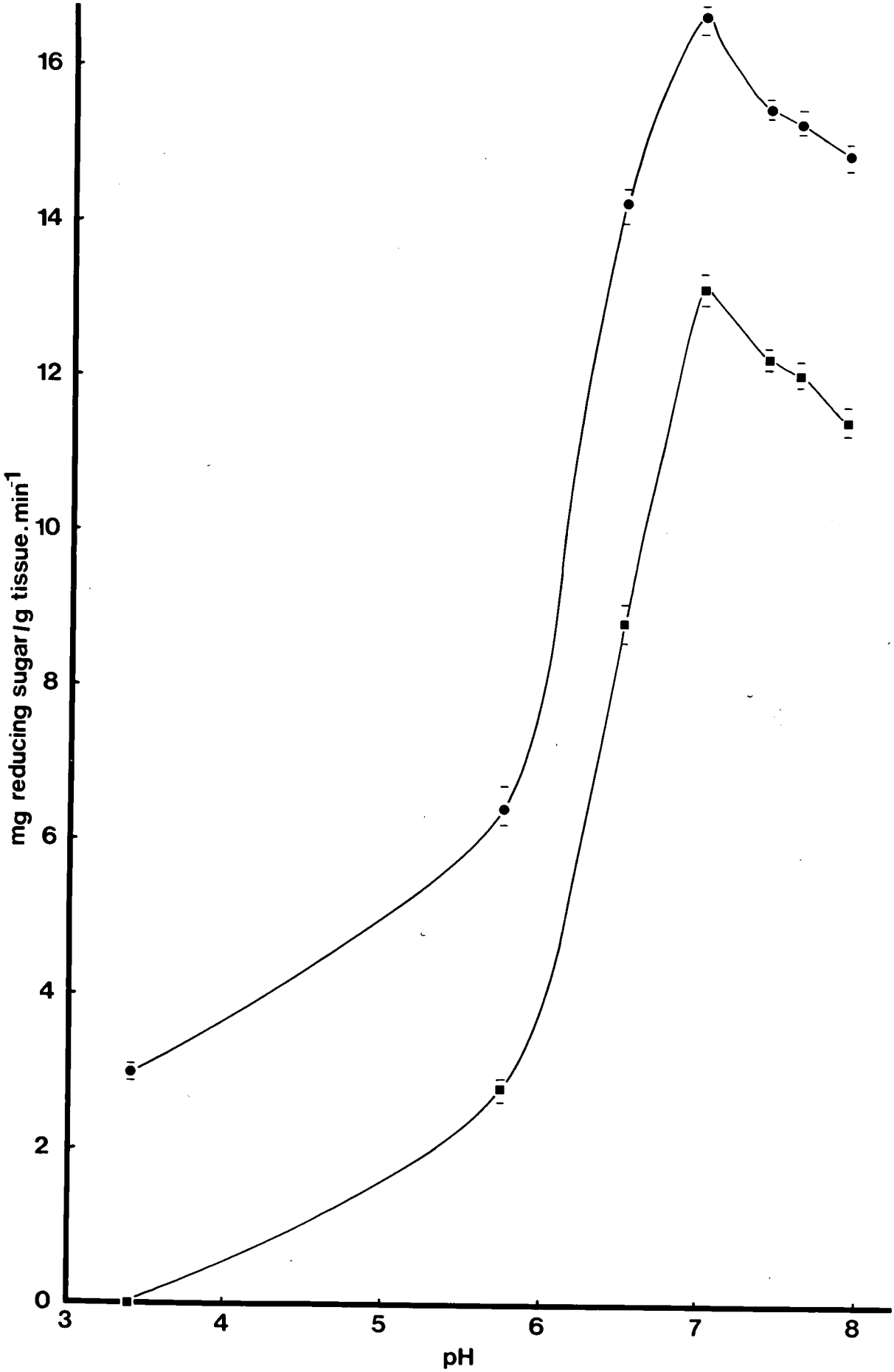
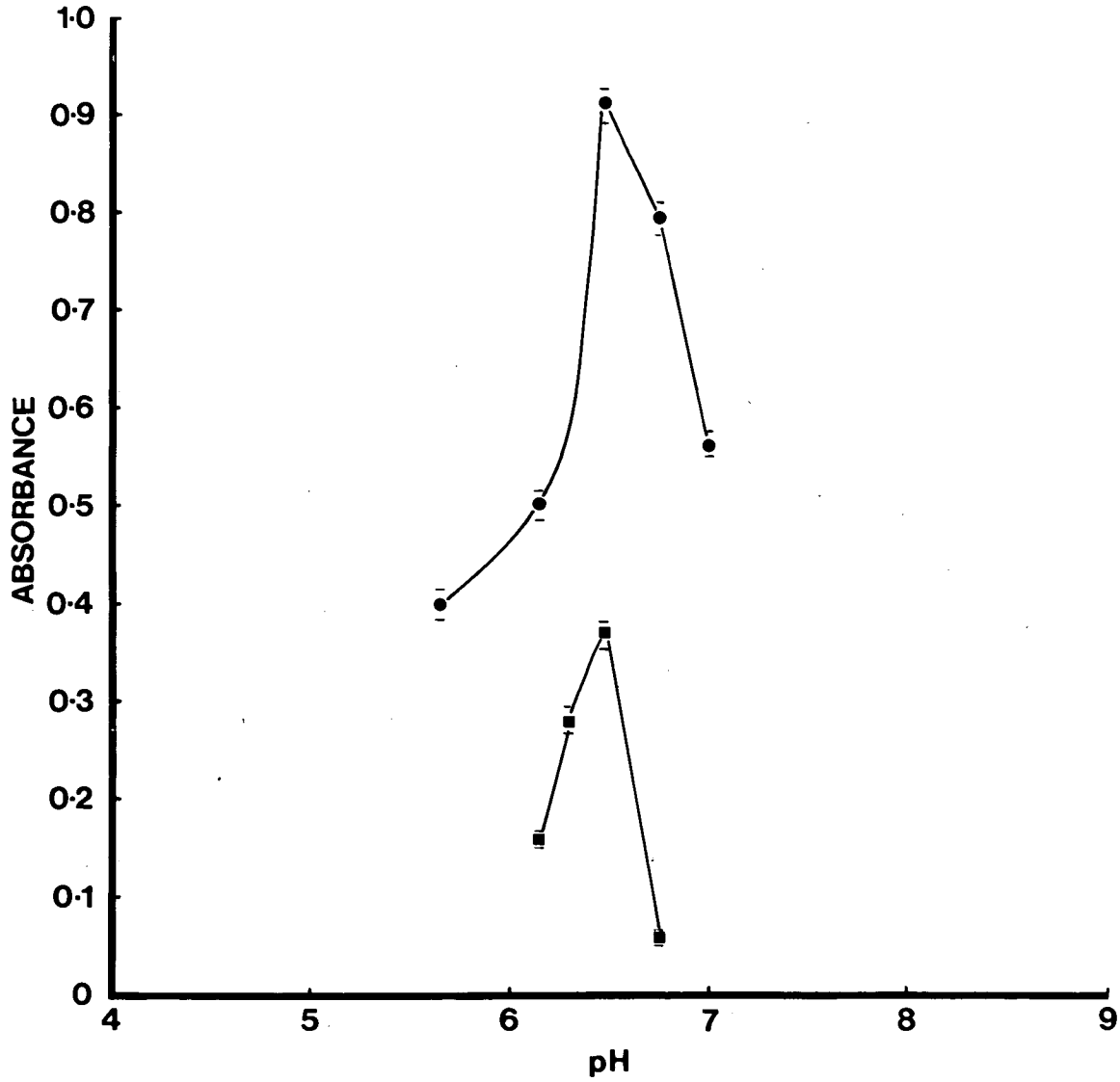


Figure 5.8 Effect of pH on chitinase activity of midgut gland (■) and stomach (●) extracts of *Parastacoides tasmanicus*, at 37.0 ± 0.2°C, (Mean and range of three measurements).

Figure 5.8



5.3.2 Assimilation efficiences of *Parastacoides tasmanicus* on controlled diets

Faeces were collected from crayfish which had been kept at 15°C and fed once on fresh worms equivalent to 57.1 ± 10.1 (8) mg dry weight (mean ± S.E. (N)). The faeces were dried and the energy content determined. The assimilation efficiencies of these animals were 90.7 ± 0.9% (8) by weight or 92.0 ± 0.6% (8) in terms of energy units. The amount of food consumed by the animals did not affect the assimilation efficiencies since there is a small standard error in the assimilation efficiencies obtained despite a large variation in the amount that the animals ate. One animal, which was in the A stage of the moult cycle had an assimilation efficiency of only 81% by weight or 83% in terms of energy units, much lower than the animals in the B and C stages of the moult cycle. Due to the shortage of animals in the A stage, and the very short duration of this stage (about 7 days), it was not possible to determine whether or not there is a difference in assimilation efficiencies between animals in difference stages of the moult cycle.

The assimilation efficiencies of crayfish fed to repletion on worms for 6 consecutive days is shown in Table 5.2.

Table 5.2 Assimilation efficiencies of crayfish fed on worms for 6 days

Test temp. (°C)	Weight of worms eaten* (mg dry wt.)	Percent assimilation efficiency* Weight	Energy units
5	168.9 ± 17.0 (4)	89.6 ± 1.6 (4)	91.1 ± 2.4 (4)
15	87.2 ± 18.4 (9)	89.2 ± 0.5 (9)	89.4 ± 0.7 (9)

*Mean ± S.E. (N)

Once again, the amount of food eaten, within the limits tested, did not seem to affect assimilation efficiency. Of course, at very low food consumption rates, when the crayfish are producing almost empty peritrophic membranes,

these membranes would then become significant parts of the 'faeces', and the apparent assimilation efficiency would drop.

The assimilation efficiencies of animals fed on a variety of foods, in experiments in which the faeces were collected by the 'condom' method, are shown in Table 5.3

Table 5.3 Assimilation efficiencies of *Parastacoides tasmanicus* fed on a variety of foods.

	Percent assimilation efficiency*			
	Weight		Energy units	
Test temp. (^{o}C)	5	15	5	15
Food source				
Worms	94.0±2.0 (6)	86.0 90.0	—	—
Filter paper	74.9±6.1 (5)	74.9 74.9 76.8	78.4±6.4 (5)	83.1 80.2 78.8
Lettuce	72.0±6.2 (4)	97.1	75.2±5.5 (4)	97.7
Boiled carrot	91.4±2.4 (12)	96.2±1.5 (9)	92.3±2.2 (12)	96.7±1.3(9)
Button grass mud	90.4±2.1 (13)	77.3±3.8 (9)	#	#

* Mean ± S.E. (N), or values when N < 4
\# Not possible to calculate - see text.

The assimilation experiments with button grass mud raised some interesting points. *Parastacoides tasmanicus* will not eat mud which has been sieved to make it more homogeneous. They will only eat when they are given clumps of untouched mud. The most likely reason for this is that sieving normally removes the larger decomposing pieces of plant material. The energy content of dried, unsieved, button grass mud was 2.620 ± 0.230 (4) kJ/g or 7.820 ± 0.875 (4) kJ/ash-free g. The ash content was 66.5 ± 1.3% (4). The energy content of faeces of animals feeding on the button grass mud was 16.600 ± 0.347 (g) kJ/g or 18.650 ± 0.433 kJ/ash-free g and the ash content was 11.0 ± 1.0% (6). (These figures are for animals kept at 15^{o}C). Obviously the

crayfish are highly selective, and choose the components of the mud with a high energy content, such as decomposing plant material, and other high energy detrital material. The crayfish must be capable of separating the food from the silica sand in the mud, and they do not feed non-selectively. The assimilation of animals feeding on button grass mud cannot be calculated in terms of energy units, as the energy value of the material they eat is unknown.

5.4 Discussion

Fat-splitting enzymes are present in the digestive system of *P. tasmanicus*. There is obviously a lipase present, as triolein is hydrolysed, and there is also an esterase, as tributyrin is attacked at a very rapid rate. Brockerhoff *et al* (1970) found one enzyme in *Homarus americanus* which hydrolysed both triolein and tributyrin, so it may be appropriate to call the enzyme(s) of *P. tasmanicus* a lipase/esterase, especially as the pH optima are very similar. However, it is unlikely that in this species a single enzyme is involved since esterase activity is higher in intestinal extracts than in the stomach, whereas lipase activity is present in the stomach but appears to be absent from the intestine Table 5.1).

The lipase and esterase activities of *P. tasmanicus* are both much higher in the midgut gland than in the stomach, as also occurs in *Astacus astacus* (Kleine, 1966). However, the optimum pH of the lipases of the two animals differs quite markedly, the optimum pH of *A. astacus* lipase being somewhere in the range 5.2 - 7.0 (although there is some disagreement on this point) while that of *P. tasmanicus* is pH 8.0 - 8.5 (see Table 5.4). In general, the optimum pH estimates of the lipase and esterase of *P. tasmanicus* are higher than those for the lipase and esterase activity of other crustaceans.

The effect of temperature on the esterase activity of *P. tasmanicus* is the same as the effect of temperature on many enzymes (Loewy and Siekevitz, 1969), but differs markedly from that found by Marcuzzi and Lafisca (1977). They found that lipases from a number of litter feeding invertebrates, when acting on various substrates, gave a wide variety of temperature/activity curves. These curves ranged from ones in which activity was constant from

Table 5.4 Optimum pH of digestive lipases of some crustaceans

	Optimum pH	Source
Anostraca		
Streptocephalus dichotomus	5.4 - 6.6	Bernice (1971)
Crab		
*Thalamita crenata**	6.97	van Weel (1960)
Lobster		
Homarus americanus	7	Brockerhoff *et al* (1970)
Freshwater crayfish		
Astacus astacus	8	Kleine (1966)
" "	5.5 - 7.0	Kruger and Graetz (1928)
" "	5.2 - 6.5	Mansour-Bek (1954)
P. tasmanicus	8.0 - 8.5	This study
" " *	7.5 - 8.5	" "

* Tributyrin esterase

5 - 50°C to ones in which there was activity at one of the temperatures tested, but not at any higher or lower temperatures. It is obvious why it would be advantageous for an ectothermic animal living in a habitat with large daily temperature fluctuations to have digestive enzymes that are relatively insensitive to temperature, but the advantages of digestive enzymes with some of the other characteristics found by Marcuzzi and Lafisca are not so obvious. They did find that in all except one case, where there was measurable activity at 50°C, the activity decreased as the temperature increased above 50°C, as it did with the esterase of *P. tasmanicus*.

Parastacoides tasmanicus has considerable protease activity in the stomach and midgut gland, with one peak of activity at pH 6.0. This is in the middle of the range of pH 5 - 7 which van Weel (1970) gave as the pH range for optimal activity of crustacean proteolytic enzymes. However a number of crustaceans have been found that have pH optima for protease activity outside this range; examples are given in Table 5.5. In fact Kozlovskaya and Vaskovsky (1970)

investigated 50 species of marine invertebrates and concluded that there was a high proteolytic activity in the Crustacea, with pH optima in the alkaline region. *Parastacoides tasmanicus* has the high proteolytic activity, but the pH optimum is clearly in the acid region. It is tempting to think that this may be an adaptation to the acid environment in which *P. tasmanicus* lives, but this is unlikely. There are other crustaceans with proteases which have an optimum pH in the acid region, and yet do not live in an acid environment, and the other digestive enzymes of *P. tasmanicus* do not have pH optima that are lower than those found in other species.

Table 5.5 Optimum pH of digestive proteases of some crustaceans

	Optimum pH	Source
Anostraca		
Streptocephalus dichotomus	7.4-8.0	Bernice (1971)
Cladocera		
Daphnia sp.	7.4	Hasler (1935)
Copepoda		
Calanus finmarchius	3.6-4.0 8.0-8.5	Bond (1934)
Crabs		
Cancer borealis	3.8 8.0	Brun and Wojtowicz (1976)
C. irroratus	3.8 8.0	" " "
C. productus	8.1-8.5	DeVillez and Buschlen (1967)
Thalamita crenata	8.53	van Weel (1960)
" "	5.12-6.70	James (1968) (unpublished data cited by van Weel (1970))
Lobster		
Homarus americanus	4 7-8	Brockerhoff *et al* (1970)
Freshwater crayfish		
Astacus astacus	6-8	Degkwitz (1957)
A. macrodactylus	5.6 7.3-9.0	Shinoda (1928)
Orconectes virilis	6.0 7.5-8.5	DeVillez (1965)
P. tasmanicus	6.0	This study

The activity/temperature curve of the protease of *P. tasmanicus* (Figure 5.5) is as would be expected. In fact it corresponds fairly closely to the activity/temperature curve of the protease of *Orconectes virilis* (DeVillez, 1965). The most interesting point about it is that activity is almost independent of temperature at normal environmental temperatures, with obvious advantages to the crayfish, while at higher temperatures activity increases rapidly with increasing temperature. This temperature independence was not noticeable in the esterase activity/temperature curve.

Sather (1969) measured the amylase activities in the midgut glands of six decapod crustaceans, and found that two omnivores had much higher amylase activities than three carnivores and one herbivore. The amylase activity of the midgut gland of *P. tasmanicus* (see Figure 5.6) is similar to that of the highest activity measured by Sather, and as Sather measured enzyme activities at 37°C rather than the 25°C used here, it is probable that the amylase activity in the midgut gland of *P. tasmanicus* is considerably higher than the activity in any of Sather's decapods. As can be seen in Figure 5.6, the amylase activity in the stomach of *P. tasmanicus* is four times the activity in the midgut gland. Unfortunately Sather did not investigate the relationship between midgut gland and stomach amylase activity in the species he studied, so a comparison cannot be made with *P. tasmanicus*. Nevertheless, it is apparent that *P. tasmanicus* has a very high amylase activity in its digestive system.

The digestive amylase of *Carcinus maenas* (Blandamer and Beechey, 1964) and other crustaceans (Vonk, 1960) is usually an α-amylase so it is probable that the amylase of *P. tasmanicus* is also an α-amylase, although a β-amylase may also be present in some crustaceans (Vonk, 1960). The optimum pH of 6.3 of *P. tasmanicus* amylase is within the range of pH 5.0 - 7.8 given by van Weel (1970) as the range of pH

optima of crustacean amylases. Table 5.6 gives the optimum pH for the amylases of a number of decapods.

Table 5.6 Optimum pH of digestive amylases of some decapod crustaceans

	Optimum pH	Source
Crabs		
Cancer borealis	7.0	Brun and Wojtowicz (1976)
C. irroratus	7.0	" " "
C. maenas	7.0	Blandamer and Beechey (1964, 1966)
Diogenes bicristimanus	7.2	Nagabhushanam and Sarojini (1968)
Metapograpsus messor	8.0	Sather (1969)
Lobster		
Homarus americanus	5.2	Wojtowicz and Brockerhoff (1972), Brockerhoff *et al* (1970)
Freshwater crayfish		
Astacus astacus	5.5-5.8	Vonk (1960)
P. tasmanicus	6.3	This study

It is worth noting that the amylase of the carnivorous marine lobster, *Homarus americanus*, is weak while the amylase of the freshwater, omnivorous *Astacus astacus* is strong, as is the amylase of *P. tasmanicus*. It may be that omnivorous decapods generally have high amylase activities (see also Sather (1969) mentioned above).

The strong cellulase present in the midgut gland and stomach of *P. tasmanicus* could be produced by the animal itself or by microorganisms in its digestive tract. *Procambarus clarkii* has both an endogenous cellulase and a cellulase produced by microorganisms, both with the same optimum pH (Yokoe, 1960). The optimum pH of the cellulases of some animals are given in Table 5.7.

By no means all of the Crustacea possess a cellulase. The isopods *Porcellio* sp. and *Armadillidium* sp. definitely do not have cellulases (Nielsen, 1962) and as explained in Section 5.1, the presence of a cellulase does not necessarily mean that it will

Table 5.7 Optimum pH of the digestive cellulases of some animals

	Optimum pH	Source
Amphipod		
Orchestia gammarella	6.5	Wildish and Poole (1970)
Freshwater crayfish		
Astacus astacus	4.0-4.5	Kooiman (1964)
Procambarus clarkii	5.8	Yokoe (1960)
P. tasmanicus	7.0	This study
Molluscs	5.2-5.5	Calow and Calow (1975), de Stevens (1955)
Earthworms	5.5	de Stevens (1955)
Most animal cellulases	5-6	Gascoigne and Gascoigne (1960)

benefit the animal concerned. Although *P. tasmanicus* has a cellulase that is very active on carboxymethylcellulose as the substrate, its activity on cellulose itself is low, *in vitro*. However, part of this apparent inactivity could be overcome if the cellulose substrate was mechanically ground up into small particles, and suspended in a finely divided state in the enzyme solution, thus exposing a large surface area to the enzyme, as would occur in the stomach of *P. tasmanicus*. In addition, *P. tasmanicus* eats partially decomposed food, and it might gain some benefit from the cellulase enzymes of the decomposer organisms. In this way the amphipod, *Gammarus pseudolimnaeus* (Barlocher and Kendrick, 1975) and the isopods, *Porcellio* sp. and *Armadillidium* sp. (Nielsen, 1962) as well as other detritus feeders such as *Astacus astacus* and *Homarus vulgaris*, obtain considerable nutrition from the food they eat, although *A. astacus* does have the ability to digest cellulose by itself. All of these animals have a cellobiase in their digestive systems, and this converts the cellobiose produced by the cellulases of decomposer organisms, or by their own cellulase, into simple sugars.

Cellobiase occurs in the stomach of most detritivores (Barlocher and Kendrick, 1975), and its presence may reasonably be predicted in *P. tasmanicus* especially as the technique used to measure cellulase activity relies to some extent on the presence of a cellobiase (see Section 5.2.1.3).

Just as cellulase is important to animals that eat plant material, so chitinase should be important to animals that eat invertebrates. The chitinase in the digestive system of *P. tasmanicus* is (probably) fairly weak. Chitinase is normally present in the digestive system of crustaceans, but as with *P. tasmanicus* it is not usually very active (Elyakova, 1972). Marine fish which feed extensively on invertebrates sometimes have considerable digestive chitinase (Goodrich and Morita, 1977a, 1977b) but this chitinase is produced by microorganisms rather than by the fish itself. It is not surprising that crustaceans do not have a strong digestive chitinase, as this would cause problems with digestion of the cuticular lining of the fore- and hind-gut. The weak chitinase present would help them to obtain some N-acetyl-D-glucosamine, or its breakdown products, from arthropods in their diet.

All of the enzymes tested for in *P. tasmanicus*, with the possible exception of chitinase and native cellulase, have moderate to high activities at their optimum pH at the temperatures used for most of the tests. However, it can be seen from the temperature/activity curves of esterase and protease (Figures 5.3 and 5.5 respectively) that at environmental temperatures the activities will be much lower. To counteract this, food spends a long time in the digestive system of *P. tasmanicus* (1-3 days at 15^{o}C and 3-5 days at 5^{o}C) which gives the enzymes plenty of time to have their

maximum effect on the food. In addition, although the conditions used to measure the activities of the enzymes were adequate for mammalian enzymes, there is no guarantee that they were optimum conditions for crayfish enzymes. For example, the emulsifiers used in the measurement of lipase activity were bile salts, whereas the emulsifiers found in the gut of *Astacus leptodactylus* and *Homarus gammarus* are fatty acyl taurine and fatty acyl dipeptides (Holwerda and Vonk, 1973), while in *Cancer* sp. emulsifiers have the general structure of acylsarcosyltaurine (van den Oord, Danielson and Pyhage, 1965). What effect the difference between *in vivo* and *in vitro* conditions had on enzyme activity is unknown.

With the above points taken into consideration, it can be said in summary that *P. tasmanicus* has the moderate lipase/strong esterase activity characteristic of many decapods, a strong protease and amylase like the omnivorous decapods studied by Sather (1969), as well as a strong cellulase, but a weak native cellulase and a weak chitinase. The status of other digestive enzymes is unknown, although it is probable that a cellobiase is present.

If the digestive enzymes of *P. tasmanicus* are any guide, it could be expected that this freshwater crayfish would digest animal material very efficiently, and plant material somewhat less efficiently. Just how efficient plant digestion is would depend on the effect that the cellulase of *P. tasmanicus* has on the cellulose components of the particular material, and how effectively the enzymes can be used. Animal food is, in fact assimilated with an efficiency equal to that of carnivores. Worms were assimilated with an efficiency of over 85% (see Table 5.3), reaching as high as 95% in some cases, which is in the upper limits of the assimilation efficiencies of carnivores. The plant material, namely lettuce and boiled carrot, was also assimilated with a very high efficiency

(see Table 5.3), with assimilation efficiencies in the range of 72.0-97.1% by weight or 75.2-97.7% in energy units (mean values). This is in the range of assimilation efficiencies reached only by those herbivores that possess enzymes capable of digesting the cellulose components of plants. The observation that filter paper was assimilated with an efficiency of approximately 75% by weight or 80% by energy units (see Table 5.3) showed that, *in vivo*, the cellulase of *P. tasmanicus* is very effective at digesting cellulose.

The high assimilation efficiencies at both 15°C and 5°C indicate that, as was suggested earlier, reduced enzymatic activity at low temperatures is compensated for by the longer time that food takes to pass through the digestive system of *P. tasmanicus*.

The assimilation rates measured in the experiments reported here would all be higher than the actual assimilation rates, if there was any overestimation of the amount of food eaten or underestimation of the amount of faeces produced. There could have been a slight loss of soluble contents of plant and animal food when it was eaten, as Dagg (1974) claims to happen with many aquatic animals, but this loss was probably small, as *P. tasmanicus* does not dismember its food to any extent (at least for the food choices provided). There could have also been some slight loss of food due to the action of microorganisms, but this loss would also have been slight. The method used to collect faeces meant that there was little, if any, loss of faecal products, as both solid and soluble products were retained inside the condoms covering the abdomens of the crayfish, and for this reason the method used to collect faeces was superior to others in which only solid faecal products are collected. Volatile faecal products would have been lost from the faeces when they were dried, of course. Even when the

possible sources of error are allowed for, there is still little doubt that the assimilation efficiencies of *P. tasmanicus* feeding on many foods are of a high order, which shows that it can utilise all types of available food very efficiently.

In its normal environment *P. tasmanicus* has access to occasional animal food, and plentiful supplies of plant material and detrital material. Detrital material can be nutritious, and is an important source of food for many animals, as it can contain a high microorganism content in addition to the products of decomposition of plant and animal material (Adams and Angelovic, 1970; Calow, 1975; Rodina, 1963). In fact, the microorganisms can be an important part of, or even the main food source for some animals that ingest detritus (Baker and Bradnam, 1976; Barlocher and Kendrick, 1973; Hargrave, 1970a; Heinle *et al*, 1977; Kostalos and Seymour, 1976; Moriarty, 1975, 1976; Newell, 1965; Tenore, 1975; Yingst, 1976). However, this is unlikely to be true of the peat which forms the button grass plains, where the rate of decomposition, and probably therefore the microorganism population, is low. The assimilation of detritus by animals is usually inefficient (Hargrave, 1970b; Mason, 1970; Richardson, 1975a; Yingst, 1976) but *P. tasmanicus* assimilates button grass mud with an efficiency of over 75% (see Table 5.3). This apparent exception to the general rule can be resolved since button grass mud has a much higher organic content than most detritus, and much of this organic matter is in the form of recognizable, largely undecomposed plant fragments. If these are selectively consumed by the crayfish, then high assimilation rates are possible. This in fact appears to be the case.

In conclusion it can be said that *P. tasmanicus* has a digestive system that is capable of a very high utilization of the food available to it.

6. SEASONAL CHANGES IN METABOLISM AND BODY COMPOSITION OF *Parastacoides tasmanicus*

6.1 Introduction

Many aquatic crustaceans, and other ectotherms, that live in an environment where there are marked seasonal variations in temperature, show adaptations that minimise the effects of these variations. This compensation is often exhibited as a higher metabolic rate, usually measured as oxygen consumption, in cold-adapted animals than in warm-adapted animals when measured at the same temperature (Bullock, 1955; Carlisle and Cloudsley-Thompson, 1968; Fry, 1958; Grainger, 1958; Hoar, 1966; Precht, 1958; Prosser, 1958; Somero, 1969. See Breteler, 1975b; Edwards and Irving, 1943; Leffler, 1972; Roberts, 1975b; Scholander *et al*, 1953; Vernberg, 1974 for examples.) This means that at their normal environmental temperatures the cold-adapted animals have a higher metabolic rate than if they were warm-adapted animals cooled to the same temperature. In effect, this may lead to similar respiratory rates during both summer and winter, although the compensation usually only partly offsets the differences in metabolism caused by the lower winter temperatures.

Such temperature compensation can also be found in populations from different latitudes. For example, under the same test conditions *Uca pugilator* collected at Woods Hole in America have a higher metabolic rate at low temperatures than *U. pugilator* collected in Florida. In this case the physiological adaptation has become fixed as a genetic adaptation, as acclimation at 20°C of animals from both populations does not abolish the difference (Démeusy, 1957).

Although some compensation is common, there are cases where no compensation (Bullock, 1955; Edwards, 1946; Ramamurthi and Janak, 1973; Scholander *et al*, 1953), or even reverse compensation (Berg, 1953; Hoar, 1966), have been reported. In such cases the animals

concerned have a very low metabolic rate during the colder months of the year, and may retreat into burrows or crevices and become dormant. This response is very useful to animals whose food source is only abundant during the warmer parts of the year. In the colder seasons these animals would be inactive, and their low metabolic rate would result in minimum depletion of their energy reserves, while when the weather warmed up they would become active and be able to replenish these reserves.

Parastacoides tasmanicus lives in an environment where there are marked seasonal temperature changes (see Chapter 2). Nothing is known about the normal metabolic rate of this animal (or any parastacid ~~freshwater~~ crayfish) and how it changes with the seasons, size of the animal concerned or changes in temperature. This study will provide some information on these points.

The metabolic rate of animals is also influenced by daily, as distinct from seasonal, temperature variations. These temperature changes do not always affect different sized animals to the same extent, so the Q_{10} often varies with the size of animals concerned (Chinnayya, 1974; Marsden, Newell and Ahsanullah, 1973; Newell and Roy, 1973; Rao and Bullock, 1954; Roberts, 1957a; Vernberg, 1959; Wolvekamp and Waterman, 1960). It is often found that small animals are less temperature dependent than larger animals of the same species. Q_{10} also varies from one temperature range to another (Chinnayya, 1974; Halcrow and Boyd, 1967; Kotaiah and Rajabai, 1972; Newell, 1973a; Teal, 1959; Vernberg, 1959; Winget, 1969), but this is only to be expected as constant Q_{10}'s over a wide temperature range would mean that there would be a logarithmic increase in oxygen consumption as temperature increased. Over narrow temperature ranges though, there is no guarantee that Q_{10} will decrease as the temperature rises.

Parastacoides tasmanicus will be largely insulated from extreme temperature changes so long as it remains in water at the bottom of its burrow (see Chapter 2); however it is believed that it often leaves its burrow at night (because of the rapid rate of recolonisation of empty burrows - see Chapter 2), and consequently some information on the effect of temperature changes on its metabolism would be useful to indicate how its metabolism is affected during these excursions.

Apart from temperature effects, the oxygen consumption of individuals of a species is also influenced by the size of the animals concerned. In general $VO_2 = aW^b$, where VO_2 is the oxygen consumption measured in mL O_2/h*, of an animal weighing W grams (either wet or dry weight), with 'a' and 'b' being constants that have to be determined empirically. The value of 'b' can vary considerably from one species to another, but is usually between 0.67 and 1.00 (Kamler, 1970; Wolvekamp and Waterman, 1960; Zeuthen, 1953). The value of 'b' also varies from one species to another, with changes in the test temperature (Armitage, 1962) and also with the time of year (Armitage, 1962; Ramamurthi and Janak, 1973). When 'b' varies with the test temperature the Q_{10} will be different for animals of different size. The constant 'a' has no absolute limits and it varies with the species of animal being tested and with the temperature at which the oxygen consumption is measured. In animals where there is a seasonal variation in oxygen consumption, it also varies with the time of year.

As has already been stated, one of the aims of the work reported in this chapter is to investigate the effect of size and other variables on the metabolism of *P. tasmanicus*. There are

*In Chapter 3 VO_2 was measured as mL O_2/g wet weight.h^{-1}, but for this chapter it is desirable to measure it as mL O_2/h as this simplifies manipulation and interpretation of the data.

numerous factors in addition to those mentioned above that have been shown to affect the oxygen consumption of ectotherms. For example, moulting in arthropods may be associated with oxygen consumption levels that are 50 to 1900% above normal (Skinner, 1962). In the Crustacea, changes in oxygen consumption that are associated with the stages of the moult cycle, especially pre- and post-ecdysis have been measured in crabs (Bliss, 1951, 1953; Lewis and Haefner, 1976; Lockwood, 1967; Roberts, 1957a), freshwater crayfish (Scudamore, 1947), isopods (Bulmheim, 1974) and euphausiids (Paranjape, 1967). There is little information on the effect of moult stage on the oxygen consumption of freshwater crayfish, and no information on the effect of moult stage on the oxygen consumption of *parastacid* crayfish.

Other factors which may affect oxygen consumption include starvation and/or feeding (Ansell, 1973; Marsden *et al*, 1973; McLeese and Watson, 1968; Newell, 1973b; Newell and Bayne, 1973; Roberts, 1957a; Vernberg, 1959; Wallace, 1973), time of day (Ansell, 1973; Ramamurthi and Janak, 1973; Rice and Armitage, 1974b), sex sometimes (Kotaiah and Rajabai, 1972; Rice and Armitage, 1974b) but not always (Laird and Haefner, 1976; Weymouth *et al*, 1944), stage of development (Newell, 1973b), activity (Grainger, 1958; Kamler, 1970; Newell, 1973b; Wolvekamp and Waterman, 1960) and oxygen tension (see Chapter 7). All of these factors must be taken into account when measurements of oxygen consumption are made.

The composition of the tissues of crustaceans changes during the year, but in general no uniform *seasonal* variations are apparent. Some animals have their storage organs filled to a maximum when the temperature is at a minimum, e.g. the freshwater crayfish, *Orconectes limosus* (Collatz, 1973), while other species build up lipid stores

during summer and then use these stores to tide them over winter, e.g. *Orconectes nais* (Armitage, Buikema and Willems, 1972) and the copepod, *Calanus hyperboreus* (Lee, 1974). There are, however, fairly uniform changes associated with food availability, moulting and reproduction.

Crustaceans do not lose body weight when they are starved. Rather, the tissue which is reabsorbed is replaced with water, as the volume of the animal concerned usually cannot change easily. The water content of the tissues can therefore be used as a rough index of the nutritional status of crustaceans (Dall, 1974). Other components apart from water content are also affected by starvation. *Carcinus maenas*, which exhibits reduced feeding activity in the late autumn and winter, metabolises lipids during these periods and the level of these in the body noticeably decreases (Heath and Barnes, 1970; Lawrence, 1976b). The marine lobster, *Panulirus polyphagus*, builds up large reserves of lipids in the midgut gland during part of the year and then 'disappears'. It probably becomes dormant and does not feed for a period. When it reappears the lipid stores are found to be considerably depleted (George and Patel, 1954). Marine crabs are said to use lipid and protein during starvation (Giese, 1966; Scheer and Meenakshi, 1961) while starved freshwater crabs such as *Paratelphusa hydrodromous* (Ramamurthi and Veerabhadrachari, 1975) and crayfish such as *Orconectes virilis* (Hazlett, Rubenstein and Rittschof, 1975; Jungreis, 1968) are thought to use at least some glycogen when they are starved, although there is disagreement over this point (Speck and Urich, 1969).

Major changes in the composition of the body tissues occur during the stages of the crustacean moult cycle. The water content of the tissues is usually highest just after moult, and lowest during late intermoult and into pre-moult (Baumberger and Olmstead, 1928;

Martin, 1973; Stein and Murphy, 1976). Lipid, which is the predominant organic reserve of many crustaceans plays an important part in the moult cycle. The lipid content of the midgut gland, and often other tissues (including the haemolymph), increases as the moult approaches, and reaches a maximum in one of the early D stages (D_0 - D_2) (Ando *et al*, 1977; Bollenbacher, Borst and O'Connor, 1972; Collatz, 1973; Kanazawa *et al*, 1976; Lautier and Lagarrigue, 1976; O'Connor and Gilbert, 1968, 1969; Spindler-Barth, 1976). In the late pre-moult the stored lipid is mobilized to meet the energy demands of all of the processes that result in ecdysis, and to act as constituents of new tissues (O'Connor and Gilbert, 1968, 1969; Teshima and Kanazawa, 1976). As a consequence of this the lipid levels start to decrease, and continue to decrease into the A and B stages after ecdysis. For example, the total midgut-gland lipid of female *Pachygrapsus marmoratus* increases steadily from 11.9% dry weight in the A stage of the moult cycle up to a maximum of 42.4% in the D_0 stage, after which it decreases to 17.8% in the D_2 stage and returns to the A stage level after the moult (Lautier and Lagarrigue, 1976).

There are also changes in the levels of organic substances, other than lipids, associated with moulting. For example, N-acetyl-glucosamine is reabsorbed from the old skeleton during pre-ecdysis, and is stored as specific proteins and polysaccharides for reuse after ecdysis in chitin production and as an energy source (Herz-Hubner and Urich, 1973; Speck and Urich, 1972). Glucose, glycogen and other polysaccharides accumulate in certain tissues, especially the midgut gland, during pre-moult, and these substances are used as reserve material for the building of chitin and mucopolysaccharides in the new skeleton (Malaczynska-Suchcitz, 1949; Scheer and Meenakshi, 1961; Spindler-Barth, 1976; Travis, 1955). Glycogen normally reaches a maximum at, or just after the moult,

after which its concentration drops, and it may disappear completely, only to appear again as a storage product when feeding commences (Lawrence, 1970; Scheer and Meenakshi, 1961; Telford, 1974; Zandee, 1966). Serum protein levels may also rise before moult, and drop again after it (Barlow and Ridgway, 1969) whereas free amino acid levels may decrease during pre-moult as these materials are used to build the new exoskeleton (Yamaoko and Skinner, 1976).

Although, as pointed out above, most crustaceans build up considerable organic stores before ecdysis, there are exceptions. *Carcinus maenas*, for example, stores less material than other crabs such as *Cancer*, and sometimes it moults without apparently having accumulated any appreciable reserves (Heath and Barnes, 1970).

Sexual reproduction in the crustaceans, especially with regard to the growth of ovaries in females, and to a lesser extent the growth of testes in males, results in changes in body composition. For example, the spent gonads of a mature female *Metapenaeus affinis* may weigh approximately 0.15 g wet weight, but when the gonads are ripe they weigh approximately 4.15 g, over 27 times the 'spent' weight. At the same time as the ovaries are increasing in weight, their water content is decreasing and the lipid content per unit weight rises (Pillay and Nair, 1973). The gonads of male prawns increase in weight from 0.04 to 0.24 g as the mating season approaches.

The eggs of marine invertebrates, including crustaceans, contain 14 - 27% lipid, as percent of dry weight, on the average (Giese, 1966). Higher levels are found but are considered exceptional. For example, the eggs of the marine copepod, *Euchaeta japonica*, contain 64.4% dry weight of lipid (Lee, Nevenzel and Lewis, 1974).

Apart from lipids, ripening ovaries may take in quantities of

protein and in some cases glycogen (Pillay and Nair, 1973). The midgut gland appears to be the source of at least some of the lipid required by the ovaries, as its lipid level often drops during the development of the ovaries (Armitage, Buikema and Willems, 1972, 1973; Clarke, 1977; Collatz, 1969; Pillay and Nair, 1973). There does not, however, appear to be any significant storage of organic substances prior to the breeding season for transfer to the tissues later.

Many mature decapods reproduce at least once per year. In crabs the period from initiation of gonad growth to spawning is about 4-7 months, e.g. *Barytelphusa cunicularis* (Diwan and Nagabhushnam, 1974). *Cherax destructor*, an Australian freshwater crayfish can breed 2 or even 3 times in one summer if kept in favourable conditions (B. Mills, pers. comm). Moulting in adult temperate-zone crayfish, and probably many crabs occurs once or twice a year. Between them, breeding and moulting are the major sources of energy expenditure of crustaceans, apart from continuing day to day energy needs.

In brief it can be said that "In crustaceans the hepatopancreas" (midgut gland) "serves as a storage depot of organic reserves and as a centre of intermediary metabolism" from which "resources are mobilized to meet the needs in moulting and reproduction" (Adiyodi and Adiyodi, 1970).

Nothing is known about the changes in the tissue components of parastacid crayfish with respect to the stages of the moult cycle or the reproductive state. The situation could be especially interesting in *Parastacoides tasmanicus* as the females carry the eggs and juveniles from April to the following summer, sometimes as late as February, and this leaves very little time for the animal

to moult before it becomes berried again, which would impose a very heavy strain on the animal's energy reserves. There is a suggestion that the animals might breed only once every two years, as only 50% of the females are berried in any year (see Chapter 2). A study of the changes in the components of the tissues of male and female *P. tasmanicus* with respect to moult stage and breeding state should elucidate the manner in which breeding, moulting and tissue components are interrelated, and should show how long the breeding cycle is, and how *P. tasmanicus* differs from, and how it is similar to, astacid crayfish and other crustaceans in respect to these matters.

It is possible to measure the different organic components of an animal's tissues, and obtain some indication of the percentage of storage materials (i.e. lipid levels higher than about 5% dry weight (Giese, 1966), glycogen etc.) and structural materials (i.e. protein, chitin etc.). Another way to study the tissues is to measure their energy content, usually in terms of joules per gram dry weight, or joules per gram ash-free dry weight, or their equivalents. This in effect converts all the organic components to a common unit, and also measures the energy content of components that may not be measured in the specific analyses, such as some sugars, nucleotides etc.

Energy measurements can provide an index to the nutritional conditions of animals. High values indicate well fed animals or animals with supplies of lipid for overwintering or egg production (Slobodkin and Richman, 1961). Except when they have been starved or when they are storing material, animals have tissues that average about 20·90 kJ/g dry weight. When storage material, usually lipid, is present, the energy value may increase to 25·00 - 29·00 kJ/g dry

weight (Richman and Slobodkin, 1960). The energy value for whole animals varies somewhat more than the values for the tissues on a dry weight basis. This is because animals may have a large amount of inorganic substances in supportive structures such as exoskeletons in the invertebrates. Therefore, values for whole animals are usually given on an ash-free dry weight basis, often with a reference to the ash content as a percentage of the dry weight.

Prus (1970) collected data on the energy content of 63 species of aquatic animals and found a range of 17·58 - 28·46 kJ/g ash-free dry weight, with 37 species falling within the range of 21·77 - 25·10 kJ/g ash-free dry weight, and a mean of 23·44 kJ/g ash-free dry weight. Prus stated that high values are found in those stages (of an animal's life) which precede a period of food shortage, or that of diminished inflow of energy from the habitat, or in organisms ready for a conspicuous outlay of energy in reproduction, (or moulting?) These values are, of course, affected by developmental stage, sex, physiological condition and other factors. For example, fresh eggs of *Eupagurus bernhardus* have a high lipid content and hence energy content (26·34 ± 1·62 kJ/g ash-free dry weight) as the young animal does not feed until it reaches the zoea larval stage, by which time its energy content has dropped considerably to 22·10 ± 2·10 kJ/g ash-free dry weight (Pandian and Schumann, 1967).

Energy values for crabs have been given as 18·40 kJ/g ash-free dry weight (51% ash) by Golley (1961). However these values are probably somewhat low as he did not allow for the 'water of hydration' (see Section 6.2.3) in the measurements of the weights of his "dried" samples. He also dried his samples at 100°C, which may have caused the loss of some of the more volatile lipids. The energy value of the freshwater crayfish, *Cambarus robustus*, of various sizes was 13·67 ± 1·97 kJ/g dry weight (18·85 ± 2·61 kJ/g

ash-free dry weight, 27.6% ash), while the energy value of adult *Cambarus immunis* was 16·38 kJ/g dry weight. These values are higher than those for crabs, but lower than those for Cladocera, Copepoda, Cirripedia, Isopoda, Amphipoda and Euphausiacea (Prus, 1970). One of the highest values recorded must be that of large, fatty, winter *Daphnia pulicaria* which have an energy content of 37·70 kJ/g dry weight (Snow, 1972).

In addition to energy values the water content of tissues is also important. For example, a tissue with 85% water content, 10% lipid and 5% protein would have the same energy content in J/g dry weight as a tissue with 70% water, 20% lipid and 10% protein, although the tissues are obviously different if the water content is also considered.

Measurements of the energy content of the tissues of male and female *P. tasmanicus*, caught at various times of the year, were used to supplement the measurements of the organic components of the tissues, and to supply additional data on the relationships between moulting, reproduction and tissue composition. They were also used to permit comparisons to be made between *P. tasmanicus* and related animals, such as the Northern Hemisphere freshwater crayfish.

6.2 Materials and methods

Animals were collected as described previously (see Section 2.2) and transported to the laboratory, where they were maintained at the water temperature measured on the button grass plains at the time of collection. As soon as possible after they had been collected a number of animals had (1) their oxygen consumption or (2) the composition of their body tissues or (3) the energy content of their tissues, measured.

6.2.1 Measurement of oxygen consumption of *Parastacoides tasmanicus*

Animals of various sizes were placed singly into flat-topped jars, of between 500 and 1500 mL capacity, the largest animals in the largest jars. The jars were carefully filled with aerated, treated Lake Pedder water (see Chapter 3) at a temperature of 5, 10, 15 or 20°C. The jars were left open for several hours, in a water bath in a constant temperature room at the appropriate temperature, to allow the animals to settle down and reach a steady state of oxygen consumption. (Ectotherms transferred from one temperature to another need time to overcome "shock" which often causes a temporary aberration in the oxygen consumption of the animals concerned, according to Grainger (1956, 1958)). The water was then gently mixed, and a sample was taken so that the oxygen content could be determined by the micro-Winkler technique (Fox and Wingfield, 1938). The jars were closed with perspex lids, with the exclusion of all air bubbles, and returned to the constant temperature rooms, where they were kept in darkness and not disturbed for 24 hours. The oxygen content of the water in the jars was then measured again, and if the oxygen content was low enough to affect the oxygen uptake of the crayfish (see Chapter 7), the results from the test concerned were not used. The animals were removed from the jars,

wiped dry and weighed. The oxygen consumption of each animal, in mL O_2/h was calculated.

In all cases only animals that had been caught within the week prior to the experiments were used for oxygen consumption measurements. It was found that for many animals held in the laboratory for longer periods, oxygen consumptions varied excessively and randomly from those of 'fresh' animals.

The linear regression of 'log oxygen consumption' on 'log weight' was determined for each group of animals that had been collected on the same date and had had their oxygen consumption measured at the same temperature. The calculations were performed on a Hewlett Packard 9825A calculator. The regression equation $\log VO_2 = \log a\ (\pm\ S.E.) + b\ (\pm\ S.E.) \log W$ was converted to the form $VO_2 = a\ (+\ S.E., -\ S.E.)W^{b(\pm\ S.E.)}$

The animals used in these experiments were neither starved nor restrained, nor forced to remain active, as measurements of the routine (spontaneous activity) oxygen consumption rate were required rather than measurements of the standard (minimum activity) rate or the excited (maximum activity) rate.

By examination of the a and b values obtained from animals caught at different times of the year, and tested at a number of temperatures, it is possible to determine whether or not *P. tasmanicus* compensates for temperature changes that occur during the year, and how weight affects the oxygen consumption of the animals. With VO_2 values from various sizes of animals at two temperatures it is possible to determine Q_{10} values, which indicate how sensitive the animals are to temperature changes, and it is also possible to determine how size affects the sensitivity of the animals.

One of the factors which determines the oxygen consumption of

crustaceans is the stage of the moult cycle. *Parastacoides tasmanicus* spends most of the year in the B and C stages of the moult cycle (see Section 6.3.1) and there appears to be little difference between the oxygen consumption of animals in these two stages. However, during the D to early B stages of the moult cycle there is a great deal of variation associated with the stages of the moult cycle. Therefore, animals that were discovered to be in the D stages of the moult cycle when they were captured (moult stages were determined by the method of Mills and Lake (1975)), had their oxygen consumption measured at intervals until they reached the early B stages of the moult cycle.

6.2.2 Measurement of organic components of the major body tissues

The total lipid, protein, glycogen and water content of the chelae muscle, abdominal muscle and midgut gland of adult male and female *P. tasmanicus* (3 - 6 g weight) were determined. In addition, the lipid, protein, glycogen and water content of the gonads of females, and attached eggs or young on berried females, were also determined. The gonads of males were too small to use for any of these determinations. To measure the composition of juveniles the whole bodies had to be used, as individual tissues were too small. The chitin content of the 'remainder', that part of the body of adults not used for other determinations i.e. mainly the exoskeleton and digestive tract, was also measured.

Each animal was washed, dried with paper towelling, weighed and sexed, and a small piece of the margin of one of its uropods cut off. These pieces were later examined and the moult stage of each of the animals determined.

The animals were dropped into hot (80^{o}C) water for a few seconds, after which the dead animals were wiped dry and dissected.

The midgut gland, muscle of the chelae, abdominal muscle plus gonads and eggs where present, were removed from each animal and placed on pre-weighed pieces of aluminium foil. The 'remainder' was placed in a 100 mL, pre-weighed flask, once any undigested food had been removed from the stomach and gut of the animal. The samples were weighed on a microtorsion balance to the nearest 0.01 mg. Portions of each of the samples, usually 20 - 40 mg, were placed in centrifuge tubes containing 10 mL of ice-cold 0.2M NaOH (for protein determination, see below) or 1 mL of 5.4M KOH (for glycogen determination, see below). The exact weights were determined by differences in the weights of samples on the aluminium foil before and after the portions were removed. The parts of the organs that remained were dried in a vacuum oven at 60^{o}C over silica gel for 7 days (for lipid determination, see below). The 'remainder' was weighed to the nearest 0.1 mg and 50 mL of 2.5M NaOH were added to it (for chitin determination, see below). Juveniles were homogenized and then treated in the same manner as each of the organs of the adults.

6.2.2.1 Protein determination

Protein determinations were carried out by the acetonylacetone method described by Golterman (1969). Each of the tissue samples in 10 mL of 0.2M NaOH was homogenized, left for several hours and then filtered through a hard-paper filter or a membrane filter. 2.0 mL samples of the filtrate were mixed with 2.0 mL of acetonylacetone reagent (2.0 mL of acetonylacetone added to 100 mL of 1 M Na_2CO_3 just prior to use) in 25 mL test-tubes, which were stoppered with marbles and heated in a boiling-water bath for exactly 15 minutes. The tubes were cooled and 14.0 mL of ethanol and 2.0 mL of Ehrlich's reagent (1.33 g of p-dimethylaminobenzaldehyde

dissolved in a mixture of 50 mL methanol and 50 mL of 12N hydrochloric acid) were added to each one. The mixtures were stirred well and then left for 30 minutes, after which the absorbances at 530 nm were measured in a Cecil 292 spectrophotometer. A standard protein solution of 1 mg/mL of 'Hammarsten' casein in 0.2M NaOH was used to make a series of standards in the range of 0 - 2.0 mg protein/2.0 mL sample. These standards were treated in the same way as the samples, and a standard curve was constructed from which the protein content of the samples could be determined.

6.2.2.2 Glycogen determination

Glycogen was measured by a modification of the methods of Giese (1967), Hassid and Abraham (1957) and van Handel (1965). Each of the test-tubes containing the tissue samples and 1 mL of 5.4M KOH was stoppered with a marble and placed in a boiling-water bath for 20 minutes. The digests were cooled and 0.1 mL of saturated Na_2SO_4 solution was added to each one, followed by the addition of 1.25 mL of 95% ethanol. The contents of the tubes were mixed and gently brought to the boil again, then cooled and centrifuged for 15 minutes at 3000 rpm. Supernatant liquid was carefully removed from each test-tube with a Pasteur pipette, and the glycogen remaining was redissolved in 1 mL of distilled water. Glycogen was reprecipitated with ethanol and Na_2SO_4, centrifuged again and the supernatant removed as before. The precipitate was redissolved in exactly 5.0 mL of distilled water.

Aliquots of 2.5 mL of solution were transferred to clean test-tubes. Standards of 0 - 50 µg of glucose in 2.5 mL of distilled water were also prepared from a 50 mg/L stock solution, which was made just minutes before it was needed. The test-tubes were placed in an ice-water bath and 5.0 mL of anthrone reagent (0.2 g anthrone in 100 mL of 95% sulphuric acid, prepared 4 hours before use and

kept in the dark until needed) were added to each one and mixed with the glycogen solution. The cold test-tubes were stoppered with glass marbles and heated for 10 minutes in a boiling-water bath, then rapidly cooled in cold water and their absorbance measured at 620 nm in a Cecil 292 spectrophotometer. The concentration of glycogen in each sample was determined from the standard curve. The determined concentrations were divided by 1.11, which is an empirical factor needed to convert the glycogen to glucose equivalents.

6.2.2.3 Lipid determination

Lipid concentrations were measured by the method of Barnes and Blackstock (1973). The tissues dried *in vacuo* over silica gel at 60°C for 7 days were weighed and then ground up with a pestle and mortar. The lipid was extracted from each sample with a 2:1 chloroform:methanol mixture; 2 - 5 mg of dried tissues were used for each 1.0 mL of solvent (2 mg tissues/mL were used for gonads, midgut gland and eggs; 5 mg tissue/mL were used for the other tissues). The slurry was filtered through a Whatman number 1 filter paper, and the residue was washed with a small quantity of solvent. 0.2 volumes of 0.15*M* aqueous NaCl were shaken with the filtrate in a small separating funnel, after which the mixture was allowed to stand overnight at 4°C. The lower phase was separated from the upper phase (which was discarded) and the volume of the lower phase was adjusted to that of the original crude extract with the addition of chloroform.

Aliquots of 0.5 mL of the extracts were transferred to Pyrex test-tubes. Into other test-tubes 0 - 0.5 mL of a standard solution of 80 mg cholesterol/100 mL of chloroform-methanol solution (equivalent to 100 µg total lipid/mL) was placed and made up to 0.5 mL with chloroform-methanol solution. The 0.5 mL aliquots

were evaporated to dryness *in vacuo*, and 0.5 mL of concentrated sulphuric acid was added to the residue of each. The tubes were plugged with non-absorbent cotton wool and heated for exactly 10 minutes at 100°C in a boiling-water bath, then cooled in cold water. Sub-samples of 0.2 mL of acid digest were transferred to clean, dry test-tubes, and 5.0 mL of vanillin reagent (13 mM vanillin in 11.8M orthophosphoric acid) were added to each one and thoroughly mixed with it. The test-tubes were allowed to stand at room temperature for 30 - 60 minutes before the optical density at 520 nm was measured in a Cecil 292 spectrophotometer. The lipid concentrations were calculated from the standard curve.

6.2.2.4 Water content

The water content of tissues was determined from the wet and dry weights of the samples used for lipid determinations. The protein and glycogen contents were measured as percentages of wet tissue weight, and the lipid contents were measured as percentages of dry tissue weight and then converted to percentages of wet weight.

6.2.2.5 Chitin determination

Chitin was extracted from the 'remainder' by the method of Hornung and Stevenson (1971). The 'remainder' was digested in 50 mL of 2.5M NaOH at 70°C for 18 hours. The remaining deproteinized material was washed with distilled water and placed in 15 mL of 9.6M formic acid for 1 hour. The material was transferred to 2.17M formic acid for 12 hours and then washed with 95% ethanol and triturated in absolute ethanol. Finally the chitin was washed with ether, dried *in vacuo* and weighed. This technique may lose a small percentage of the chitin, but it should extract a high percentage in most cases, and therefore would be adequate for

comparisons between animals in different moult stages, and for comparisons of *P. tasmanicus* with other crayfish.

6.2.3 Measurement of the energy content of body tissues

Animals were washed in water, dried, weighed and sexed, and a small piece of the uropods cut off for determination of the moult stages of the animals. The animals were then killed by immersion in hot water for a few seconds.

The midgut gland, muscle of the chelae, abdominal muscle, gonads (of females) and eggs or attached young (of berried females) were dissected out of the adult crayfish and placed on pre-weighed squares of aluminium foil. The 'remainders' were placed in pre-weighed 25 mL flasks. All of the tissues and the 'remainders' were weighed as soon as possible and then dried in a vacuum oven at 60^{o}C over silica gel until no change in weight was observed with continued drying. Juveniles were dried whole.

The dried tissues were weighed, ground up, made into pellets and then redried for 24 hours. The pellets were weighed and the energy content determined in either a Phillipson type micro-bomb calorimeter (Phillipson, 1964) or in a Gallenkamp ballistic bomb calorimeter. The weight of solid material left in the crucible of the bomb after combustion of the sample was taken as the ash weight of the sample, although some slight loss of ash from the crucible may have occurred. Acid corrections were not applied, as the error from this source is likely to be only 0.3 - 0.4% (Schroeder, 1977; cf. Kersting, 1972).

The 'remainder' and juveniles were treated slightly differently. They were dried, ground up, pelletized, redried and their energy content was determined in the semi-micro bomb calorimeter, but a number of corrections were applied to the values obtained. The main reason for these

was that the exoskeleton has a high proportion of $CaCO_3$ and other inorganic substances, whereas the other tissues do not. One correction which might have been expected, but which was not performed, was a correction to compensate for the loss of heat during combustion due to the endothermic decomposition of $CaCO_3$ (Paine, 1966). The exoskeleton has a high $CaCO_3$ content when compared to other tissues, but it is still at most only 20% of the dry weight (calculated from data in Mills and Lake (1976)), and this is low enough to make corrections for endothermy unnecessary.

Two other corrections were necessary however. Firstly, corrections were made to compensate for the presence of 'water of hydration' in the exoskeleton (Paine, 1964). A sample of the 'dried' 'remainder' or juveniles was weighed and then heated at 173^{o}C to remove the water of hydration (and hopefully little else), after which it was weighed again and the percentage of 'dried' weight which was actually water was determined.

The other correction that was needed was due to the loss of ash from the crucible of the bomb calorimeter, when the energy content of the 'remainder' of juveniles was determined, with the resultant underestimation of the ash content of the sample, if the residue in the crucible was used as a measure of the ash content (Paine, 1964). This correction is probably unnecessary for samples with less than 10% ash, but it can be important for samples with an ash content higher than this. Ash remaining in the crucible after the energy determination of the 'remainder' or juveniles was often only 20 - 30% of the initial dried weight. However, when a sample was ashed in a muffle furnace at 550^{o}C for 8 hours the ash content of the samples was found to be 35 - 45% for the 'remainder' and 25-28% for juveniles (means). The muffle furnace estimates of ash content were used both for the 'remainder' and for the juveniles.

6.3 Results

6.3.1 The moult cycle of *Parastacoides tasmanicus*

Before the results of most of the experiments are given, a few details need to be provided about the moult stages of *P. tasmanicus*. The moult stages were determined by the method of Mills and Lake (1975). It was noticed that under normal environmental conditions the duration of the various stages of the moult cycle of adults differed considerably from that found by Mills and Lake. These differences can probably be attributed to temperature differences. Mills and Lake kept their animals in the laboratory at 12 ± 1°C for over a year, while in normal conditions the animals are exposed to temperatures ranging from as low as 1°C in winter to as high as 25°C in summer (see Chapter 2). It was found in this study that the moult stages that occur during the warmer periods of the year last for a shorter time than claimed by Mills and Lake, while those that occur during the colder periods of the year last for a longer time.

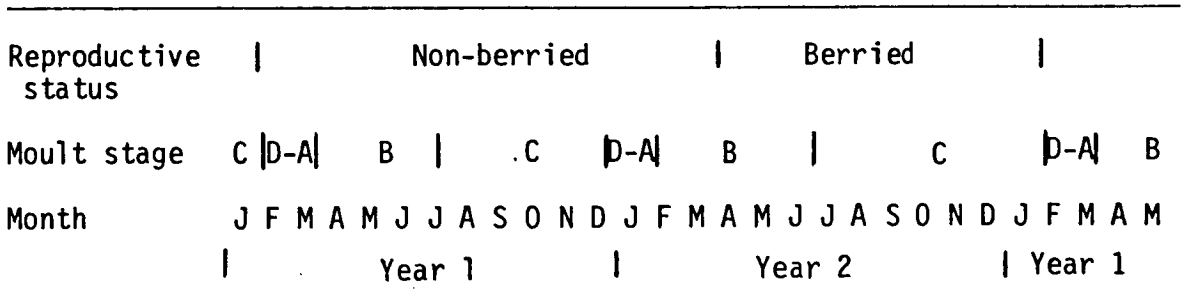
In males the D - A moult stages occur sometime during the period of October-March (mainly December-February) and take a total of about 1 month. The B stage lasts until June-July and the C stage fills the remaining time until the D stages start again. There is considerable variation between animals, and from one season to the next, as is shown by the 6 month range that the D - A stages can occur within, but as a generalisation it may be said that the B and C stages occupy the period from March until November.

The situation is more complicated with females. Berried females cannot moult until all the young have left them. Therefore they often do not moult until February-early April. Females that had developing young attached to their abdomens in February were found to still be in the C stage of the moult cycle, so it is possible

that berried females do not enter the D stage of the moult cycle until all of the young have left them. There are no berried females in March (see Chapter 2), but they occur again in April in the B stage of the moult cycle and this lasts until the end of June, when the C stage commences. This stage lasts until the females are about to moult in the following February-early April. Non-berried females are less restricted than berried females as to when they are able to moult. They normally commence the C stage sometime in July, and then enter the D - A stages sometime between December and March. Some non-berried females can be found in the B stage from January onwards.

As will be shown later, the females that were not in berry one year become the following year's berried females (in April) and vice versa. Therefore the situation can be confusing from February to April as there is no external difference between a female that was in berry the previous year but whose young have all left her, and a female that was not in berry the previous year. Figure 6.1 is an attempt to clarify the situation by diagramatically representing the two year cycle of moult stages and berried-nonberried status of adult female *P. tasmanicus*.

Figure 6.1 Reproductive and moult stage cycle in female *Parastacoides tasmanicus*



Juveniles can be found in the B or C stages of the moult cycle at almost any time of the year, and it is probable that they moult

several times in the period between November and April. Very few juveniles have been captured in the D or A stages of the moult cycle, and it is probable that these stages are even shorter in the juveniles than they are in adults.

6.3.2 Seasonal variation in oxygen consumption

Table 6.1 gives the dates of capture of animals, the temperature of their burrows on the button grass plains at a depth of 20 centimetres, the temperatures at which oxygen consumption was measured, the regression equations relating weight to oxygen consumption, some Q_{10} values for 1 and 5 g animals over some temperature ranges at various times of the year, the F values from the analysis of variance tables of the regression results, and P(F), i.e. the probability of the F value being as large as it is if there is no significant difference between regression and residual variance. In 3 cases P(F) was greater than 0.05, in 4 more cases P(F) was between 0.01 and 0.05, while in the remaining 12 cases P(F) was less than 0.01. The data from the experiments where P(F) was greater than 0.05 were not used for any further calculations, and where P(F) was between 0.01 and 0.05 the data was only used when no 'better' data were available.

The oxygen consumption of males and non-berried females only were measured to derive the regression equations. There is no statistically significant difference between the oxygen consumption of these two classes whereas the oxygen consumption of berried females does seem to differ. However, the number of berried females was inadequate to satisfy other demands for them and still have their oxygen consumption studied extensively. One experiment in which the oxygen consumption of 10 berried females was measured, followed by removal of the eggs and then another measurement of the females' oxygen consumption a day later gave results with such a large 'residual'

Table 6.1 Oxygen consumption and Q_{10} obtained for *Parastacoides tasmanicus* under different conditions

	Capture date	Burrow temperature (°C)	Test temperature (°C)	VO_2 (mL O_2/h) $a(+S.E.,-S.E.)W^{b(\pm S.E.)}$	Q_{10} (temperature range) 1 g animal	5 g animal	N	$F_{v1,v2}$*	P(F)
SUMMER	17/2/'76	15	5	$0.016(+0.011,-0.007)W^{0.667(\pm0.295)}$	3.9 (5-15°C)	3.3 (5-15°C)	7	$F_{1,5}$= 5.1	P>0.05
			15	$0.057(+0.015,-0.012)W^{0.683(\pm0.165)}$			8	$F_{1,6}$=17.2	P<0.01
	30/3/'76	15	5	$0.014(+0.003,-0.003)W^{0.829(\pm0.140)}$			9	$F_{1,7}$=35.0	P<0.01
			15	$0.055(+0.009,-0.008)W^{0.730(\pm0.096)}$			10	$F_{1,8}$=58.3	P<0.01
AUTUMN	28/5/'75	7.5	5	$0.028(+0.007,-0.006)W^{0.472(\pm0.180)}$	1.5 (5-15°C)	1.9 (5-15°)	18	$F_{1,16}$=6.9	0.05>P>0.01
			15	$0.043(+0.014,-0.010)W^{0.601(\pm0.230)}$			16	$F_{1,14}$=6.8	0.05>P>0.01
	30/5/'76	7.5	10	$0.056(+0.030,-0.013)W^{0.576(\pm0.176)}$			18	$F_{1,16}$=7.1	0.05>P>0.01
WINTER	9/8/'75	5	5	$0.015(+0.002,-0.002)W^{0.993(\pm0.070)}$	3.6 (5-10°C)	2.8 (5-10°C)	17	$F_{1,15}$=202.7	P<0.01
			10	$0.029(+0.003,-0.003)W^{0.918(\pm0.069)}$	1.9 (10-15°C)	1.5 (10-15°C)	17	$F_{1,15}$=249.4	P<0.01
			15	$0.039(+0.003,-0.003)W^{0.847(\pm0.054)}$	1.1 (15-20°C)	1.6 (15-20°C)	16	$F_{1,14}$=199.1	P<0.01
			20	$0.042(+0.004,-0.004)W^{0.954(\pm0.068)}$	2.6 (5-15°C)	2.0 (5-15°C)	16	$F_{1,14}$=179.5	P<0.01
	13/8/'76	5	5	$0.021(+0.006,-0.005)W^{0.540(\pm0.173)}$	2.0 (5-15°C)	2.2 (5-15°C)	12	$F_{1,10}$=9.8	0.05>P>0.01
			15	$0.042(+0.013,-0.010)W^{0.609(\pm0.188)}$			12	$F_{1,10}$=10.5	P<0.01
SPRING	24/10/'75	6.5	5	$0.025(+0.006,-0.005)W^{0.613(\pm0.154)}$	2.5 (5-10°C)	3.3 (5-10°C)	8	$F_{1,6}$=15.8	P<0.01
			10	$0.038(+0.009,-0.007)W^{0.687(\pm0.151)}$			8	$F_{1,6}$=20.7	P<0.01
			15	$0.059(+0.013,-0.010)W^{0.427(\pm0.128)}$			4	$F_{1,2}$=11.1	P>0.05
	23/11/'76	13	5	$0.011(+0.009,-0.005)W^{0.420(\pm0.400)}$			8	$F_{1,6}$=1.1	P>0.05
			10	$0.020(+0.001,-0.001)W^{0.617(\pm0.045)}$	5.4 (10-15°C)	7.2 (10-15°)	7	$F_{1,5}$=187.8	P<0.01
			15	$0.046(+0.003,-0.003)W^{0.708(\pm0.047)}$			8	$F_{1,6}$=225.6	P<0.01

*v1 and v2 are the degrees of freedom

variation that the results were unacceptable. It seems likely though that the oxygen consumption of berried females is lower than that of other animals.

It is fairly obvious that there is little acclimatization to seasonal changes in environmental temperatures by *P. tasmanicus*. This can be seen more easily in Table 6.2. There is no increase in 'a' values at a constant test temperature of 15°C, as the environmental temperature drops, as would be expected to happen if acclimatization was occurring. In fact, if there is a change it is that 'a' values decrease as the environmental temperature does. The value of 'a' for August 1975 is significantly lower than the value for March 1976, (t-test:$0.05 > P > 0.025$) but there is no significant difference between any of the other values, although the lower values occur in the colder months and the higher values occur in the warmer months. The 'a' values at a test temperature of 5°C show no apparent trend. There is almost no difference between the 'a' values for March 1976 and August 1975, but they are both less than the value for October 1975, the difference between August 1975 and October 1975 being statistically significant (t-test: $0.025 > P > 0.01$). (There is also a significant difference between the 'b' values for these two months which would tend to counteract the difference in oxygen consumption caused by the difference in 'a' values.)

Table 6.2 Regression variables for the weight:oxygen consumption relationship of *Parastacoides tasmanicus*

Approximate environmental temperature (°C)	Month	Test temperature (°C)	Regression variables (means) a	b
15	Feb. '76	15	0.057	0.683
15	March '76	15	0.055	0.730
5	August'75	15	0.039	0.847
5	August'76	15	0.042	0.609
13	Nov. '76	15	0.046	0.708
15	March '76	5	0.014	0.829
5	August'75	5	0.015	0.993
6.5	Oct. '75	5	0.025	0.613

There may be a slight acclimatization to temperature changes through the year, with respect to the 'b' values, but this acclimatization would be more apparent in large animals than in smaller ones. There is a tendency for 'b' values to be higher in the cooler months than in the warmer months (see Tables 6.1 and 6.2) although there is only the one significant difference between the 'b' values mentioned above. If larger samples provide statistical support for this tendency it would mean that in the cooler months the value of W^b, (and therefore the oxygen consumption) would be higher than if the 'b' values for the warmer months were still applicable. This difference would partly offset the lack of change (or even decrease) in the 'a' values at the lower temperatures, but it would advantage larger animals more than smaller ones, as a change in 'b' has a greater effect on the value of W^b when W is large than when it is small.

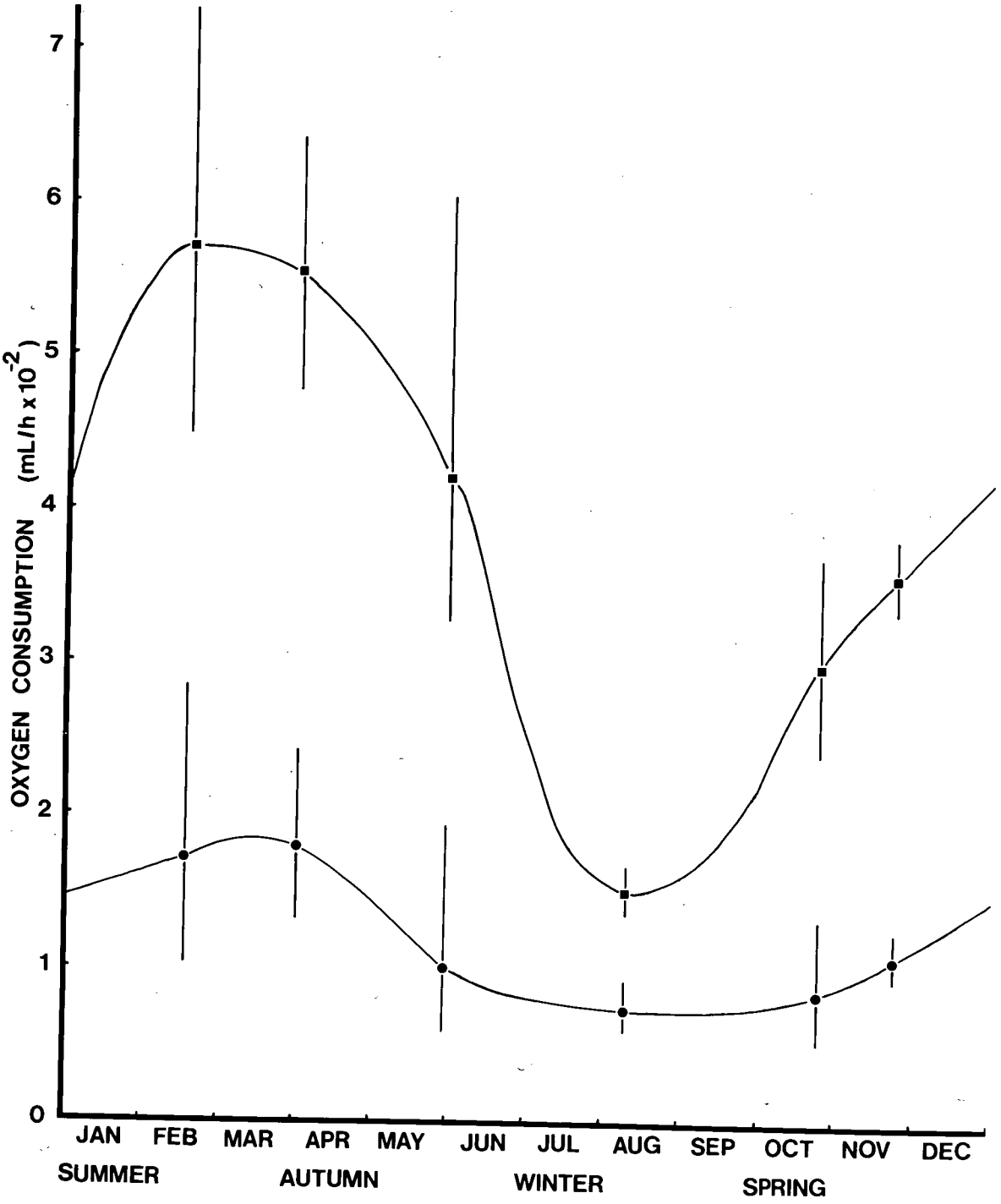
The results of these interactions are displayed graphically in Figure 6.2, which illustrates the calculated oxygen consumption of theoretical 1 g and 5 g *P. tasmanicus* at environmental temperatures during the course of a year. The small animals have a respiration rate in February-March that is 3.8 times the respiration rate in August. Adults are not quite so greatly affected by seasonal temperature changes (for the reasons mentioned above) but the respiration rate in March is still 2.4 times the minimum rate, which occurs in August.

Very little can be said about the Q_{10} values, with assurance, as there are not enough reliable estimates. There is a strong suggestion that the values are higher in the warmer months for the 5^{o} - 20^{o}C range. Whether or not the pattern is similar in summer is not known. How Q_{10} varies with animal size is not definitely known, as the values increase with animal size

Figure 6.2 Calculated oxygen consumption for theoretical *Parastacoides tasmanicus* of 1 g (■) and 5* g (●) weight at environmental temperatures, during the year. (Mean ± S.E.).

* x 10^{-1}

Figure 6.2


OXYGEN CONSUMPTION (mL/h x 10^{-2})
0
1
2
3
4
5
6
7
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
SUMMER
AUTUMN
WINTER
SPRING

in some cases and decrease with increasing animal size in other cases. The variation is, of course, due to changes in the 'b' values from one test temperature to another, and the way that these alter from one season to the next.

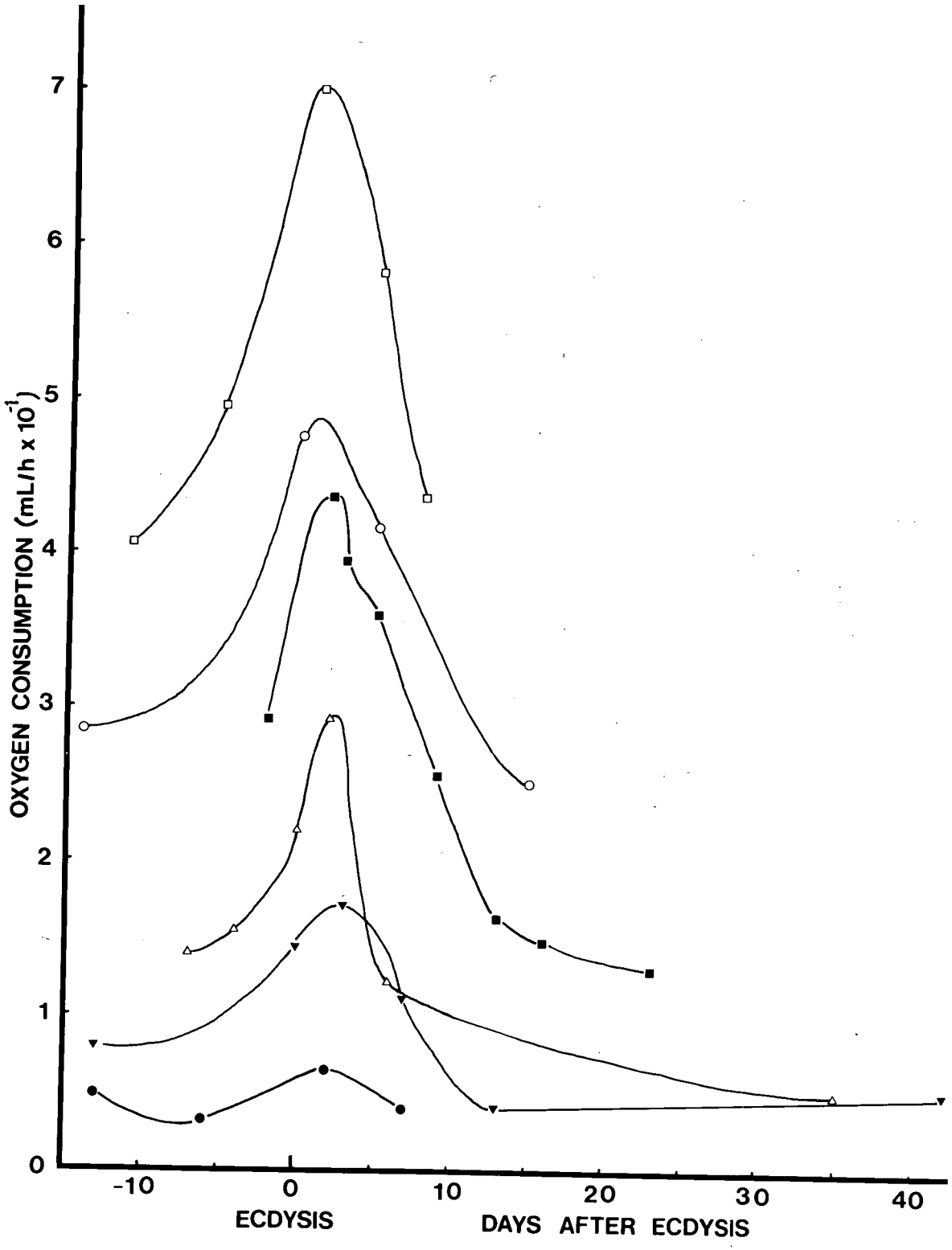
Most crustaceans increase their oxygen consumption prior to ecdysis and maintain it at a high level until some time after ecdysis. *Parastacoides tasmanicus* is no exception. Figure 6.3 shows the oxygen consumption of 6 animals at various times before and after moult,i.e. the D to early B moult stages, at 15^{0}C, in the laboratory. These animals showed little of the random variation in oxygen consumption that animals in the B and C stages showed when they were kept for long periods in the laboratory, so the changes in oxygen consumption with moult stage were not masked. During the week prior to ecdysis, the late D stages of the moult cycle, the oxygen consumption increased steadily, and continued to increase until it reached a peak one to three days after ecdysis, in the A moult stage. The oxygen consumption rate decreased steadily over the next three weeks, and eventually reached a steady state, which was as low as 25% of the peak level in some cases. By this time the animal was in the B stage of the moult cycle.

It is interesting to note just how much the oxygen consumption of *P. tasmanicus* varies during the year. Animals in the B and C moult stages were used in the measurements of oxygen consumption during the year, and as mentioned above, these animals had an oxygen consumption in summer that was 2 to 4 times the winter rate. When it is realized that this summer rate can be doubled or tripled (or more) during ecdysis, it can be seen that the routine metabolic rate of *P. tasmanicus* can vary by a very large amount in the course of a year.

Figure 6.3 Oxygen consumption of 6 *Parastacoides tasmanicus* before, during and after ecdysis, at 15°C, in the laboratory.

Symbol	Initial weight of crayfish (g)	Sex
□	10.37	Female
■	9.82	Male
○	7.71	Male
▼	3.10	Male
△	3.05	Female
●	0.836	Juvenile

Figure 6.3


OXYGEN CONSUMPTION (mL/h x 10⁻¹)
0
1
2
3
4
5
6
7
-10
0
10
20
30
40
ECDYSIS
DAYS AFTER ECDYSIS

6.3.3 Organic composition of the body tissues

6.3.3.1 Females

It was found very early in this study that the composition of the tissues of *P. tasmanicus* is very dependent on the moult stage, sex and reproductive state, i.e. berried or non-berried in females, of the crayfish. Table 6.3 shows the organic composition of adult female crayfish. The females are divided into berried and non-berried (and one animal 'just out of berry') and these two groups are further divided, not by the time of year that the animals were collected, but rather by their moult stage.* Unfortunately no freshly caught females in the A or D stage of the moult cycle were available for use in this experiment, so there is only data on females in the B and C stage of the moult cycle.

It was shown in Chapter 2 that at no time of the year are more than 50% of the female crayfish over 3 g weight in berry, and that females could breed no more than once a year. The following argument using the data provided in Table 6.3 and Figure 6.1 shows that females only breed once in every *two* years.

The gonads of berried females in the B stage of the moult cycle are understandably small (1 - 2 mg), as they have just been 'spent' to produce the eggs that the females carry. However, even towards the end of the year, and into the beginning of the new year, when the berried females are in the C stage of the moult cycle, and the eggs have hatched and the juveniles have started to desert their mothers, the gonads of these females are still very poorly developed (10.4 ± 3.0 mg). Even as late as February (females become berried in April), females that were in berry during the previous months

* Due to the small number of animals available, animals in the B and C stages of the moult cycle come from a time span of several months. The values are therefore not necessarily typical of the beginning or end of the moult stage concerned, but may be considered to represent values in the middle of the stage.

Table 6.3 Percentage composition of body tissues of female *Parastacoides tasmanicus* in different reproductive states and moult stages. (Mean ± S.E.).

	Females in berry				Females not in berry		Female just out of berry
Moult stage (number)*	B (3)			C (8)	B (6)	C (7)	C (1)
Animal wet weight (g)	4.11, 5.38, 5.63			5.11 ± 0.28	5.10 ± 0.46	4.88 ± 0.24	3.90
Tissue							
Chelae muscle							
Weight (mg)	129.1	241.3	320.9	264.1 ± 43.4	246.4 ± 24.4	260.0 ± 37.5	438.4
Water (%)	80.85	81.00	82.16	78.26 ± 0.79 (7)	77.10 ± 0.97 (5)	77.61± 0.99 (6)	81.40
Protein (%)	6.22	8.05	8.61	8.60 ± 0.66	7.79 ± 0.46	7.90± 0.54	7.12
Glycogen (%)	0.10	0.32	0.91	0.38 ± 0.06	0.51 ± 0.14 (5)	1.15± 0.40	0.13
Lipid (%)	0.45	0.52	1.63	1.06 ± 0.18	0.84 ± 0.10	0.78± 0.09	0.49
Abdominal muscle							
Weight (mg)	238.7	256.00	369.8	227.4 ± 21.5	234.8 ±17.4	267.8 ± 31.7	188.6
Water (%)	82.40	84.35	84.40	81.77± 0.57 (7)	79.06 ±1.42 (5)	78.06± 1.64 (6)	83.59
Protein (%)	5.70	5.74	7.77	7.20± 0.70	6.95 ±0.43	6.42± 0.34 (6)	9.42
Glycogen (%)	0.29	0.99	1.33	0.58± 0.19	1.04 ±0.34 (5)	1.13± 0.35 (6)	0.11
Lipid (%)	0.53	0.80	1.44	1.30± 0.18	1.04 ±0.13	0.98± 0.16	1.00
Midgut gland							
Weight (mg)	169.8	266.5	-	227.3 ± 17.8	193.9 ±22.8	214.0 ± 16.3	160.8
Water (%)	75.00	75.07	77.80	71.38 ± 1.51 (7)	63.83 ±6.25 (5)	67.71± 2.46 (6)	76.66
Protein (%)	7.29	8.32	9.34	8.09 ± 0.55	7.39 ±0.71	7.49± 0.43 (6)	8.48
Glycogen (%)	0.23	1.12	2.01	0.83 ± 0.16	1.10 ±0.17	0.95± 0.19	0.41
Lipid (%)	3.65	4.24	5.88	8.89 ± 1.27	10.57 ±1.32	14.68± 2.87 (6)	2.15
Gonads							
Weight (mg)	1 - 2			10.4 ± 3.0	80.3 ±17.7	80.1 ±12.1	18.2
Water (%)	-			78.28 ± 1.58 (5)	49.48 ±8.98 (5)	46.03± 2.38 (6)	69.78
Protein (%)	-			4.85 (1)	6.78 ±1.05 (5)	7.04± 0.54 (6)	-
Glycogen (%)	-			0.62 (1)	0.65 ±0.14 (5)	0.83± 0.13	-
Lipid (%)	-			8.00 ± 1.09	25.29 ±3.01	28.03± 1.23	13.38
Eggs							
Weight (mg)	50.7	82.4	-	171.2 ± 32.4 (6)			
Water (%)	48.05	61.55	62.76	68.93 ± 5.07(6)			
Protein (%)	4.80	6.07	6.85	5.83 ± 0.82(7)			
Glycogen (%)	0.56	0.74	1.15	0.50 ± 0.13(7)			
Lipid (%)	15.76	22.30	27.09	16.44 ± 3.17(7) #			
Remainder							
Weight (g)	2.51	3.72	3.83	3.31 ± 0.21(7)	3.42 ±0.32 (5)	3.38 ± 0.62 (5)	2.51
Chitin (%)	3.34	4.42	4.57	4.83 ± 0.21(7)	5.05 ±0.14 (5)	4.73 ± 0.45 (5)	4.07
Tissues + Remainder (Percent initial weight)	78.64	80.54	-	83.72 ± 1.24(7)	85.17 ±1.69 (5)	86.42 ± 2.00 (5)	85.03
Body - (Tissues + Remainder) (%)	19.46	21.36	-	16.28 ± 1.24(7)	14.83 ±1.69 (5)	13.58 ± 2.00 (5)	14.97

* Numbers are also shown in brackets when values are given for less than the whole sample of animals.

#3 animals had eggs with more than 23% lipid whereas 3 other animals had eggs with less than 10% lipid.

still have small gonads; this is demonstrated by the 'just out of berry' female whose gonads only weighed 18.2 mg. It would obviously be impossible for the gonads of these recently berried females to develop quickly enough to allow the females to again become berried by April of the same year.

Instead, the females moult and enter the B stage as non-berried females (see Figure 6.1). Since they do not have to protect or carry around a brood of eggs, they can move around and eat better than berried females, and the gonads can grow during the remainder of the warmer months, so that by the time the C stage is reached in the middle of the year, the gonads have already reached their maximum size of 80.3 ± 17.7 mg (see Table 6.3). During this period the water content of the gonads has dropped from 78% (in the stage berried females) to 49%, while the lipid content has increased from 8% to over 25% of the wet weight of the gonads. The difference is again statistically signifcant (t-test : $P<0.05$). In the C stage non-berried females the lipid content of the gonads increases further, from 25% to 28%, so that by the end of this stage the gonads of the non-berried females are of sufficient size, and have enough energy stored to provide the eggs of the berried females in the following April. It is useful to note the high degree of similarity in weight and lipid content of the gonads of C stage non-berried females to the fresh eggs of B stage berried females (especially on a dry weight basis).

At the same time as the gonads are increasing in size and lipid content, there is an increase in the lipid content of the midgut gland, with a concomitant reduction in water content. B stage berried females have midgut glands containing only 4.6% lipid by weight. This increases to 8.9% in the C stage berried females, to 10.6% in the B stage non-berried females and to 14.7% in C stage non-berried females. The difference between the lipid content of the C stage berried females and C stage non-berried females is statistically significant (t-test : $P<0.05$). Not only do C stage non-berried

females have much larger gonads, with a higher energy content per unit weight, than do C stage berried females, they also have a much larger store of lipid in their midgut glands.

Concomitant with the obvious (and significant) changes mentioned above, there are some less obvious differences between berried and non-berried females. Although the protein content of the tissues of the animals is so variable that no definite trends can be detected, there appear to be differences and trends in the lipid, glycogen and water content of the various tissues, apart from the ones already mentioned.

Thus, glycogen levels in the tissues of the B and C stage non-berried females are, with one exception, higher than the levels in the corresponding tissues and moult stage of the berried females. The mean glycogen content of the midgut gland, the muscles of the chelae and the abdominal muscle is significantly higher in non-berried females than in berried females (t-test : $P < 0.01$). Lipid levels in the tissues of the berried females appear to increase slightly from the B stage to the C stage, and then drop slightly, except in the midgut gland, to the levels of the B stage non-berried females. The sample numbers are too low to show any statistically significant changes, but if the above interpretation is supported by larger samples, it would be reasonable to suppose that transfer of lipid occurs from muscle tissues in the chelae and abdomen to the midgut gland and/or gonads during the D-A and the B stages of the non-berried females. This transfer continues during the C stage in the non-berried females.

As would be expected, the water content of the muscle tissue from the chelae and abdomen of berried females decreases from the B stage to the C stage ($P < 0.05$), following the increase that occurs during the intake of water and increase in size during ecdysis. However, the water content of these tissues has actually decreased even further by the time the animal has moulted again and reached the B stage as a non-berried female, but does not decrease appreciably from the B stage to the C stage in the non-berried females. In other words, despite the massive amounts of lipids that are being

incorporated into the gonads and midgut gland during this period, the animals are able to moult, but afterwards have a lower water content in all tissues as B stage non-berried females than they had as C stage berried females. Moulting is usually an energy-expensive process (involving the uptake of a large amount of water to increase the volume of the animal concerned (see for example Dall and Smith (1978)). In the present case this is clearly not so. Furthermore there is no further decrease in water content from the B to the C stage of non-berried females, unlike that which occurs in the tissues of the berried females.

If attention is given to the changes that occur when the C stage non-berried females moult and then become the B stage berried females, it can be seen that some remarkable changes take place. Apart from the expected decrease in the weight of the gonads due to the production of eggs, there is a decrease in the lipid content of the midgut gland from 14.7% of the total wet weight of the gland in the C stage non-berried femaels to 4.6% in the B stage berried females, with a concomitant increase in the water content from 68% to 76%. There are also increases in the water content of the chelae and abdominal muscles (from 78% to 81% in the chelae and from 78% to 84% in the abdomen), and a decrease in the percentage of the body that is composed of tissue and 'remainder', from 86.4% to 79.6%. This indicates that the haemolymph volume (much of which is lost during dissection of animals and is estimated from the difference between initial weight and tissues + 'remainder') of the body has increased by an average of 6.8%. These changes are all in sharp contrast to the changes that take place when C stage berried females moult and eventually become B stage non-berried females.

One possible explanation that can be offered to explain these differences is that the moult of females 'just out of berry' does not involve any increase in body size, as these animals cannot 'afford' to use their already meagre energy stores in this way, and need to channel all their effort into building up the gonads and midgut gland. On the other hand,

the moult of the non-berried females could be accompanied by an increase in size, as there is no need to transfer any more lipid to the gonads, and there are large energy reserves in the midgut gland.

6.3.3.2 Males

The composition of the tissues of male *P. tasmanicus* in the various moult stages is shown in Table 6.4. Unfortunately only one animal was caught in each of stages D_2, A and A-B of the moult cycle, and so differences between these stages and the other stages of the moult cycle cannot be examined statistically. However, the 'trends' can still be examined to see if they show the anticipated changes, although their validity cannot be tested without larger numbers of animals. For example, it seems that the lipid levels and to a lesser extent the glycogen levels in the midgut gland and other tissues, increase to a high level just before moult, and then decrease rapidly during the A stage and into the early B stage. This agrees with the normally accepted idea that animals build up energy stores before moulting and then utilise these stores during the period of rapid growth and tissue development that follows immediately after moulting. It is also noticeable how the protein levels fall before the moult, especially in the muscles of the chelae, presumably due to tissue degeneration which makes it easier for the animal to remove itself from the old exoskeleton. After the moult the protein levels increase rapidly in the late A - early B stages, coinciding with the reduction in the levels of glycogen and lipid.

Lipid levels increase during the B stage to a level that is maintained during the C stage (Table 6.4). These levels are comparable to those in the B and C stages of the non-berried females, as is the water content of the tissues, while the protein levels might be slightly higher in male tissues in these moult stages. The haemolymph volume, as roughly estimated from the percentage of the initial body weight which is neither tissue nor "remainder" is lower in all moult stages of the male than in any of the stages in the berried female, and is similar to the values for non-berried females. It seems that male crayfish and female non-berried crayfish are roughly identical

Table 6.4 Percentage composition of body tissues of male *Parastacoides tasmanicus* in different moult stages. (Mean ± S.E.)

Moult stage (number)*	A (1)	A-B (1)	B (8)	C (10)	D_2 (1)
Animal wet weight (g)	8.72	3.55	4.55 ± 0.44	4.69 ± 0.52	5.63
Tissue					
Chelae muscle					
Weight (mg)	917.0	152.7	306.5 ± 54.5	349.3 ± 41.5	253.9
Water (%)	82.67	81.52	76.31± 2.01 (7)	78.75± 0.98 (9)	-
Protein (%)	4.35	10.10	9.43± 0.58	7.31± 0.35	4.42
Glycogen (%)	1.22	0.08	0.48± 0.18	0.94± 0.28	0.37
Lipid (%)	0.76	0.60	0.81± 0.14	0.74± 0.14	1.34
Abdominal muscle					
Weight (mg)	1132.6	530.5	236.1 ± 15.1	234.3 ± 21.1	274.9
Water (%)	80.4	82.69	78.12± 2.74 (7)	79.86± 1.02 (9)	-
Protein (%)	5.65	8.00	7.59± 0.29	6.71± 0.43	5.36
Glycogen (%)	1.47	0.03	0.68± 0.24	1.15± 0.29 (8)	1.37
Lipid (%)	1.04	0.77	0.95± 0.13	0.92± 0.13	1.33
Midgut gland					
Weight (mg)	405.1	150.8	193.3 ± 16.5	198.5 ± 31.4	282.5
Water (%)	70.66	78.89	65.66± 3.78 (7)	72.29± 2.05 (9)	-
Protein (%)	4.45	6.96	9.20± 0.51	8.17± 0.48	7.34
Glycogen (%)	0.76	0.44	0.91± 0.22	1.00± 0.18	1.11
Lipid (%)	17.28	2.68	10.21± 1.83	10.21± 2.13	21.54
Remainder					
Weight (g)	4.89	2.25	3.03± 0.34 (7)	3.21± 0.43 (8)	-
Chitin (%)	2.14	3.31	5.74± 0.08 (7)	5.37± 0.15 (8)	-
Tissues + Remainder (Percent initial weight)	84.23	86.87	85.59± 0.73 (7)	86.92± 1.28 (8)	-
Body - (Tissues + Remainder) (%)	15.77	13.13	14.41± 0.73 (7)	13.08± 1.28 (8)	-

* Numbers are also shown in brackets when values are given for less than the whole sample of animals.

Table 6.5 Percentage composition of juvenile *Parastacoides tasmanicus* in different moult stages. (Mean ± S.E.)

Moult stage (number)	Just off mother*	B (11)	C (5)	D_1'''-D_2 (1)
Animal wet weight (mg)	8.55	271.2 ± 40.1	220.7 ± 63.9	337.6
Water (%)	78.71	71.34± 1.13	70.14± 2.24	74.00
Protein (%)	4.17	5.85± 0.19	5.90± 0.56	4.40
Glycogen (%)	0.25	1.16± 0.21	1.04± 0.32	0.69
Lipid (%)	7.65	2.10± 0.20	1.44± 0.31	1.60

* 16 animals pooled

in tissue composition, while female berried crayfish are in much 'poorer' condition than the other two. This supports the idea that berried females are not capable of moulting in the 'normal' way that the males and non-berried females do, and that even if they did manage to, they could certainly not then produce eggs in the same season.

The chitin content of the 'remainder' of the males increases from 2.1% in the A stage to 5.7% in the B stage, as the exoskeleton is formed and strengthened. The B and C stage chitin levels in the male are appreciably higher than the corresponding levels in the females (maximum of 5.1% in B stage non-berried females). This is at least partly due to the possession of larger and stronger chelae by male *P. tasmanicus*, as evidenced by the larger muscles of the chelae of the males, despite the fact that the males weighed less then the females (see Tables 6.3 and 6.4). In addition the exoskeleton as a whole appears to be slightly thicker and stronger in males (personal observation).

6.3.3.3 Juveniles

Table 6.5 shows the organic composition of juvenile *P. tasmanicus*. The smallest of these had just left their mother, while the others were small enough so that they were probably no more than 6 months old, with the B stage juveniles being younger than the C stage

Table 6.6 Energy content, wet and dry weights, water and ash content of the tissues of adult *Parastacoides tasmanicus*. (Mean ± S.E.)

Sex and Reproductive state	Males		Berried females		Non-berried females	
Moult stage (number)*	B (6)	C (5)	B (2)	C (5)	B (7)	C (6)
Animal wet weight (g)	5.47 ± 0.34 (5)	7.23 ± 0.75	6.47 -	5.77 ± 0.47	4.64 ± 0.42	6.29 ± 0.23
Tissue						
Chelae muscle						
Wet weight (mg)	366.6 ± 41.6	575.3 ± 110.1	232.7 323.9	377.5 ± 63.6 (4)	258.6 ± 42.8	301.3 ± 22.2
Dry weight (mg)	71.8 ± 11.3	132.2 ± 29.6	23.6 63.0	74.2 ± 10.6 (4)	56.1 ± 9.5	65.7 ± 5.7
Water (% wet weight)	80.5 ± 1.5	78.0 ± 1.3	89.9 80.5	80.0 ± 0.7 (4)	78.1 ± 1.0	78.3 ± 1.0
Energy (kJ/g ash-free dry weight)	24.512 ± 0.963	22.624 ± 0.418	22.849 24.161	21.844 ± 0.372(4)	22.811 ± 0.307	21.870 ±0.345
Ash (% dry weight)	7.7 ± 0.7	7.1 ± 0.8	5.6 8.2	8.0 ± 0.3 (4)	7.4 ± 0.5	7.9 ± 0.2
Abdominal muscle						
Wet weight (mg)	253.8 ± 20.5	401.1 ± 112.9	153.7 219.5	281.3 ± 19.8	242.4 ± 28.3	311.1 ± 38.2
Dry weight (mg)	43.0 ± 5.8	84.1 ± 25.5	16.8 38.0	42.4 ± 1.9	48.1 ± 8.0	62.7 ± 8.5
Water (% wet weight)	82.0 ± 2.0	79.4 ± 1.0	82.7 89.1	84.8 ± 0.5	80.3 ± 1.5	79.8 ± 1.4
Energy (kJ/g ash-free dry weight)	25.109 ± 0.478	22.196 ± 0.536	22.374 23.395	22.654 ± 0.663	23.125 ± 0.304	21.492 ± 0.461
Ash (% dry weight)	8.7 ± 0.8	5.6 ± 0.5	5.3 8.3	8.3 ± 1.2	7.3 ± 0.7	5.7 ± 0.3
Midgut gland						
Wet weight (mg)	231.5 ± 8.6	341.8 ± 37.6	215.2 231.8	252.5 ± 25.4	209.1 ± 20.1 (6)	283.0 ± 16.4
Dry weight (mg)	53.0 ± 4.3	100.8 ± 11.8	44.7 57.1	68.1 ± 4.4	64.2 ± 8.1 (6)	82.4 ± 5.1
Water (% wet weight)	75.6 ± 2.5	70.6 ± 0.8	75.4 79.2	72.4 ± 2.5	69.2 ± 3.0 (6)	70.8 ± 1.4
Energy (kJ/g ash-free dry weight)	26.206 ± 1.506	26.030 ± 0.978	23.422 24.580	24.110 ± 0.839	27.385 ± 1.126 (6)	24.629 ± 0.456
Ash (% dry weight)	5.8 ± 0.7	3.8 ± 0.5	4.6 4.8	4.1 ± 0.1	3.7 ± 0.6 (6)	4.0 ± 0.2
Gonads						
Wet weight (mg)			1 - 2	31.5 ± 8.0	68.8 ± 19.3	90.4 ± 13.8
Dry weight (mg)			-	7.1 ± 3.2	35.7 ± 12.1	39.3 ± 7.4
Water (% wet weight)			-	80.0 ± 3.2	53.9 ± 4.8	58.5 ± 3.7
Energy (kJ/g ash-free dry weight)			-	28.628 ± 0.232(4)	32.680 ± 1.352	30.136 ± 0.791
Ash (% dry weight)			-	4.3 ± 0.7 (4)	5.9 ± 1.0	4.8 ± 0.4
Eggs						
Wet weight (mg)			244.3 266.3	169.2 ± 22.8		
Dry weight (mg)			103.5 105.3	44.4 ± 8.6		
Water (% wet weight)			57.6 60.5	73.2 ± 3.0		
Energy (kJ/g ash-free dry weight)			30.307 31.906	28.644 ± 0.507		
Ash (% dry weight)			2.1 3.9	5.5 ± 0.6		
Remainder						
Wet weight (mg)	3893.5 ± 222.3	4883.0 ± 528.2	4267.6 4704.0	3666.5 ± 330.1	3209.4 ± 290.4	4312.8 ± 145.3
Dry weight# (mg)	1283.5 ± 102.2	1729.6 ± 218.1	1025.5 1250.1	993.0 ± 101.1	984.8 ± 90.2	1322.6 ± 42.0
Water# (% wet weight)	67.0 ± 2.0	65.0 ± 1.4	73.4 76.0	73.0 ± 1.0	69.2 ± 1.1	69.3 ± 0.9
Energy# (kJ/g ash-free dry weight)	22.372 ± 1.808 (5)	21.506 ± 1.743	21.007 21.715	21.283 ± 2.256 (4)	22.516 ± 1.617 (6)	21.998 ± 0.749 (5
Water of hydration (% Remainder dry wt.)	9.8 ± 1.1 (5)	11.7 ± 1.2	7.5 10.9	10.5 ± 1.5 (4)	8.6 ± 1.0 (6)	9.8 ± 0.7 (5
Ash# (% dry weight)	45.2 ± 2.0 (5)	43.7 ± 2.5	29.4 45.4	41.9 ± 1.0 (4)	42.3 ± 3.4 (6)	40.2 ± 2.0 (5

* Numbers are also shown in brackets when values are given for less than the whole sample of animals.
\# Dry weight includes water of hydration, but this is taken into account when energy is determined i.e. Ash and energy are determined after the water of hydration has been removed.

juveniles. There is little that can be said about the results except to note the large lipid reserves still contained by the juveniles that have just left their mother, and to note the drop in lipid content from the B to the C moult state. There also appears to be a drop in protein content and an increase in lipid, but not glycogen in the animal preparing to moult. These data cannot be compared with the data for the adults as the entire juveniles were used in the determinations rather than separate tissues as for the adults.

6.3.4 Energy content of the body tissues

Table 6.6 shows the wet and dry weights, water content, energy content and ash content of the tissues and 'remainder' of adult male, and berried and non-berried female *P. tasmanicus* in the B and C moult stages. Table 6.7 shows the total body wet and dry weight, water content, energy content and ash content of juvenile *P. tasmanicus*. These juveniles were considerably larger than the ones discussed in Section 6.3.3.3 and were probably up to 1 year old.

Table 6.7 Energy content, wet and dry weights, water and ash content of juvenile *Parastacoides tasmanicus*. (Mean ± S.E.)

Moult stage (number)	B (5)	C (9)
Body wet weight (mg)	793.9 ± 32.9	511.1 ± 89.9
Body dry weight † (mg)	206.3 ± 81.2	136.2 ± 26.9
Water † (% wet weight)	74.2 ± 2.4	74.1 ± 1.3
Energy †(kJ/g ash-free dry weight)	25·436 ±0·832	22·059 ±0·907
Water of hydration (% dry weight)	9.2 ± 0.1	9.0 ± 1.8
Ash † (% dry weight)	27.5 ± 3.9	25.4 ± 1.5

† Dry weight includes water of hydration, but this is taken into account when energy is determined i.e. Ash and energy are determined after the water of hydration has been removed.

The water content and tissue weight data support most of the ideas proffered in Sections 6.3.3.1 and 6.3.3.2. In the females there is a progressive increase in gonad size in the moult stages from B stage berried females to C stage non-berried females. There is also an increase in lipid content of the gonads and midgut gland, as indicated by the increase in energy (joules per gram ash-free dry weight) and decrease in water content. The water content in the tissues of both males and females varies to about the same extent as reported in Sections 6.3.3.1 and 6.3.3.2.

The energy levels in the tissues are mostly within the range of 21·00 to 24·00 kJ/g ash-free dry weight, most of the exceptions being those tissues possessing high lipid levels. When ash and water of hydration are measured by the correct methods even the values for the 'remainder' fall within this range.

The ash content of the chelae and abdominal muscles of both males and females varies between 5.6% and 8.7% with no readily discernible pattern. The ash content of the midgut gland is lower, and varies between 3.7% and 5.8%. The higher levels in the muscle are likely to be due to the presence of calcified apodemes to which the muscles are attached. The low levels in the midgut gland suggest that this organ is unlikely to be used as a site for the storage of inorganic salts, at least not in the stages studied. The ash content of the eggs and gonads is also low (3.0 - 5.9%), while the juveniles have a much higher ash content (25.4 - 27.5%).

The differences in the ash content of the 'remainder', which is mainly exoskeleton, between the males and the females supports the view that the exoskeleton of males is stronger than that of females. The ash content is higher in males than in females, while the water content is lower. This view was further supported by observations made on specimens that were dissected. The exoskeleton of males,

especially the carapace and chelae, was noticeably more difficult to cut and less flexible than that of females.

The juveniles cannot be compared directly with the adults, as whole juveniles, rather than tissues, had their calorific values determined. The values found for juveniles in the B stage were slightly higher than all adult tissues except for those of the midgut gland, while C stage juveniles had an energy content that fell within the ranges found for adult tissues.

In addition to the results given above, a few facts were gathered from crayfish that died shortly after moulting, after they had been kept in the laboratory for some time. For one of these the dry weight of exuvia was 18% of the pre-ecdysis dry weight of the crayfish (1.78 g), while for the other it was 22.1% of the pre-ecdysis dry weight of 1.09 g. Both of these crayfish were males. The energy value of the exuvium of the first crayfish was 15·87 kJ/g ash-free dry weight, while the ash content was 57.5% of the dry weight. The energy content of the post-ecdysis crayfish was 24.03 kJ/g ash-free dry weight with an ash content of 47.6% of the dry weight.

Several crayfish that had recently moulted were observed in the laboratory and it was estimated that they consumed over three quarters of the exuvium, and assimilated it with an efficiency of approximately 80-90%. They did not eat some of the harder parts of the exuvium, and this, plus the incomplete digestion resulted in a loss of about 30% of the energy in the exuvium. How this high assimilation rate was achieved, considering the low chitinase activity, is unknown.

6.4 Discussion

Parastacoides tasmanicus exhibits little in the way of metabolic adaptation to compensate for seasonal temperature changes, as evidenced by the large annual variation in oxygen consumption shown in Figure 6.2. This is not a rare phenomenon, but unlike most other 'non-compensators', *P. tasmanicus* still remains reasonably active in winter despite its low oxygen consumption rate. In cold weather it still moves around above ground to some extent, as was shown by the rate of reoccupation of empty burrows in mid-August to mid-October (see Chapter 2).

The 'b' values in the equation $VO_2 = aW^b$ vary considerably among crustaceans, but are normally within the range of 0.67 - 1.00 (see for examples Aldrich, 1974; Bulmheim, 1974; Chinnayya, 1974; Ellenby, 1951; Laird and Haefner, 1976; Leffler, 1973; Lewis and Haefner, 1976; McLeese and Watson, 1968; Richman, 1958; Roberts, 1957a; Simmons and Knight, 1975; Small, Hebard and McIntire, 1966; Weymouth *et al*, 1944; Wycliffe and Job, 1977). Most of the values for *P. tasmanicus* are in this range, so there is nothing unusual in that respect. However, when the oxygen consumption rates of *P. tasmanicus* and other decapod crustaceans are compared, some large differences can be seen. Table 6.8 shows the weight-specific oxygen consumption rates (aW^{b-1}), test temperature (normally near normal environmental temperatures), weight and source of data for a number of decapod crustaceans. It is apparent that, at its normal environmental temperature, *P. tasmanicus* has a weight specific oxygen consumption that is somewhat lower than that of most other decapod crustaceans at their respective environmental temperatures. It is even lower than that of the large, relatively sedentary, benthic, palinurid and homarid lobsters. In many cases the difference is greater than shown in Table 6.8, if the weight of the animals

Table 6.8 Oxygen consumption of some decapod crustaceans

	Weight (g)	Temp. (°C)	Oxygen consumption (mL $O_2/g.h^{-1}$)	Source
Hermit crabs				
Pagurus splendescens	-	0	0.020-0.042	Scholander *et al* (1953)
" "	-	10	0.040-0.090	" " " "
True crabs				
Cancer magister	650 - 1150	8 - 10	0.0366	Johansen, Lenfant and Mecklenburg (1970)
Callinectes sapidus	200	20 - 27	0.0856	Batterton and Cameron (1978)
Carcinus maenas	21 - 70	16	0.0693	Arudpragasam and Naylor (1964)
" "	41.5 - 80	10	0.0378	Hughes, Knights and Scammell (1969)
Chionoecetes opilio	757	5	0.0144	McLeese and Watson (1968)
Libinia emarginata	-	37	0.45	Vernberg and Vernberg (1968)
Paratelphusa hydrodromous	10	15	♂0.045 ♀0.040	Kotaiah and Rajabai (1972)
Pugettia producta	5	15	0.123	Weymouth *et al* (1944)
" "	10	15	0.106	" " " "
Burrowing shrimps				
Callianassa californiensis	5.3 ± 1.5	10	0.029 ± 0.009	Thompson and Pritchard (1969)
Upogebia pugettensis	5.7 ± 1.3	10	0.059 ± 0.014	" " " "
Spiny lobsters				
Palinurus elephas	Large	15	0.044	Wolvekamp and Waterman (1960)
Panulirus interruptus	200 - 600	13	0.034	Winget (1969)
" "		16	0.048	" "
Lobsters				
Homarus americanus	454	5	0.0146	McLeese and Watson (1968)
" "	-	12 - 15	0.072	McMahon and Wilkens (1975)
H. gammarus	-	15	0.020-0.042	Wolvekamp and Waterman (1960)
H. gammarus (H. vulgaris)	189	15	0.035	Bosworth, O'Brien and Amberson (1936)
Freshwater crayfish				
Orconectes immunis	8.4	16	0.068	Wiens and Armitage (1961)
O. nais	8.4	16	0.066	" " " "
Procambarus alleni	-	25.1	0.066	Wolvekamp and Waterman (1960)
Cambarellus shufeldtii	♂0.18 ♀0.26	29	♂0.140-0.654 ♀0.121-0.342	Fingerman (1955)
Astacus astacus	-	15	0.030-0.054	Wolvekamp and Waterman (1960)
A. leptodactylus	-	19 - 21	0.070	" " " "
Austropotamobius torrentium	-	19 - 21	0.100	" " " "
Cherax destructor	10	10	0.0336	Woodland (1967)
Parastacoides tasmanicus	1	5	0.0151(+0.0017,-0.0015)	This study
" "	5	5	0.0149(+0.0036,-0.0029)	" "
" "	10	5	0.0148(+0.0045,-0.0035)	" "
" "	1	15	0.0571(+0.0155,-0.0122)	" "
" "	5	15	0.0343(+0.0225,-0.0136)	" "
" "	10	15	0.0275(+0.0235,-0.0127)	" "

concerned is taken into account. For example, the data supplied in McLeese and Watson (1968) allows the oxygen consumption of theoretical 5 and 10 g *Homarus americanus* to be calculated. At 5°C the oxygen consumption of such animals would be 0.0244 and 0.0225 mL $O_2/g.h^{-1}$ respectively, much higher than that of a similarly sized *P. tasmanicus* at the same temperature. So, although large *Homarus americanus, H. gammarus (H. vulgaris), Chionoecetes opilio, Panulirus interruptus, Palinurus elephas* and *Astacus astacus* have weight-specific oxygen consumptions that approach that of *P. tasmanicus*, if the weight of the animals concerned is taken into consideration *P. tasmanicus* has a much lower weight-specific oxygen consumption than many of the other decapod crustaceans at normal environmental temperatures. Much of the information from Table 6.8 is shown graphically in Figure 6.4, in which log of the oxygen consumption of some decapod crustaceans (at their "normal" temperature) is plotted against weight. The oxygen consumption of *P. tasmanicus* is below that of most animals of a similar size, and falls below the recognisable 'curve' made by the plotting of log oxygen consumption of animals of different weights.

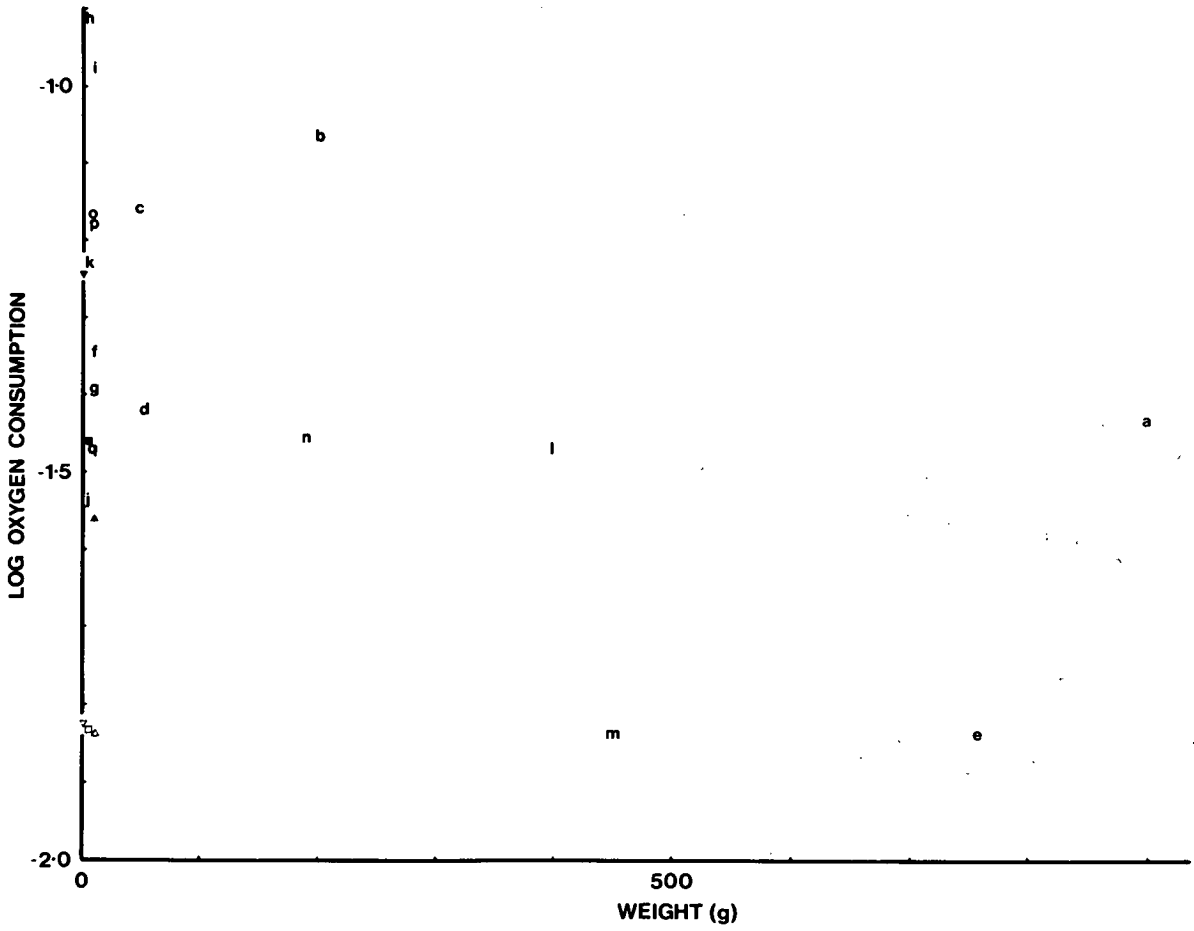
As stated in Section 6.3.2 there is not much that can be said about the Q_{10} values measured during the year for *P. tasmanicus*, as there are not enough reliable estimates. If, however, the suggestion that the Q_{10} values are higher in summer than in winter, is correct, then it would mean that the high summer oxygen consumption rates are more affected by changes in temperature than are the low winter oxygen consumption rates. The significance of this fact is not apparent, especially as the animals are shielded from much of the daily temperature fluctuations by being below ground (see Chapter 2). The other suggestion, namely that Q_{10} values decrease with increasing temperature, is not unexpected, for the reasons mentioned earlier. It is unfortunate that there are not enough reliable estimates to show what difference there is between small and large animals in the way that they are affected by temperature changes.

The increase in oxygen consumption of *P. tasmanicus* as it approaches ecdysis (see Figure 6.3) is similar to that found in

Figure 6.4 Log oxygen consumption of decapod crustaceans of different weights. Data was obtained from Table 6.8. Only mean weights and oxygen consumption are shown.

Species			Symbol
Cancer magister			a
Callinectes sapidus			b
Carcinus maenas		16°C	c
"	"	10°C	d
Chionoecetes opilio			e
Paratelphusa hydrodromous			f (♂) g (♀)
Pugettia producta	5g		h
"	"	10g	i
Callianassa californiensis			j
Upogebia pugettensis			k
Panulirus interruptus			l
Homarus americanus			m
Homarus gammarus			n
Orconectes immunis			o
Orconectes nais			p
Cherax albidus			q
Parastacoides tasmanicus	1g	5°C	▽
	5g	5°C	□
	10g	5°C	△
	1g	15°C	▼
	5g	15°C	■
	10g	15°C	▲

Figure 6.4


LOG OXYGEN CONSUMPTION
-1·0
-1·5
-2·0
0
500
WEIGHT (g)
a
b
c
d
e
f
g
h
i
j
k
l
m
n

some marine crabs (Bliss, 1951, 1953; Edwards, 1950; Lewis and Haefner, 1976; Roberts, 1957a) and one species of freshwater crayfish, *Cambarus immunis*, (Scudamore, 1947). There is a gradual rise in oxygen consumption from late intermoult until the time of moult, and a decline in postmoult stages. For example, *Pachygrapsus crassipes* has a two-fold increase in metabolism two weeks prior to moult, at the onset of the D stage (Roberts, 1957a).

However, there seems to be some difference of opinion about when the maximum oxygen consumption occurs. The oxygen consumption of *Pachygrapsus crassipes* and *Gecarcinus lateralis* (Bliss, 1951, 1953) was found to rise slowly and reach a peak a few days after moult, as was the case with *P. tasmanicus* in this study. Lewis and Haefner (1976), on the other hand, found that with *Callinectes sapidus* the highest oxygen consumption occurred during late premoult. These authors also noticed a drop in oxygen consumption at moult, which continued for about 12 hours after moult. They stated that this drop was due to a cessation of movement and a build-up of an oxygen debt due to a number of factors which made aerobic respiration difficult at that time. A sharp drop in the oxygen consumption of *Carcinus maenas* just prior to ecdysis has also been recorded (Truchot, 1964), but no such drop in oxygen consumption was noticed for the few *P. tasmanicus* that were measured during the moult, although a transient, or slight reduction would not have been detected.

Although the effect of feeding and/or starvation on the oxygen consumption of *P. tasmanicus* was not tested, all of the animals used for oxygen consumption tests were freshly caught and were supplied with food during their captivity, so results would not have been affected by differences between animals. Likewise, although the presence or absence of a diurnal variation

in oxygen consumption was not tested for, all animals had their oxygen consumption measurements started at approximately the same time, and finished at the same time so this factor did not affect results either. The sex of the crayfish did not seem to affect the oxygen consumption, although as stated earlier, berried crayfish did not have their oxygen consumption measured accurately. It is believed that berried females have lower oxygen consumptions than males or non-berried females. Of the other factors mentioned in Section 6.1 as having an effect on oxygen consumption, oxygen tension, and to a lesser extent activity will be discussed in Chapter 7. For the animals used here, activity was at a 'routine' level, i.e. spontaneous activity, and the oxygen tension was always high enough so that the oxygen consumption of the crayfish was not restricted.

The composition of the tissues of *P. tasmanicus*, as measured here, compares favourably with that observed in other Crustacea. Lipid is obviously the major energy source (see Tables 6.3 and 6.4), although glycogen does play a minor role. Changes in energy reserves in both males and females are mainly a function of the composition of the midgut gland, the main component of which, after water, is lipid.

In neither the males nor the females were there statistically significant differences in either the wet or dry weight of the midgut glands among crayfish in the various moult stages and/or nutritional states and/or stages of ovarian development (in females). However, there is a trend in the dry weight of midgut glands in females, whereby the smallest (dry weight) midgut glands are found in B stage berried females, larger midgut glands are found in C stage berried females, and still larger midgut glands

are found in B stage non-berried females. These differences may prove to be statistically significant in larger samples, in which case they would provide still further support for the hypothesis that female *P. tasmanicus* operate on a two-year reproductive cycle. In the American freshwater crayfish, *Orconectes nais*, no correlation was found to exist between the dry weight of the midgut gland and either moulting or ovarian development (Rice and Armitage, 1974a).

The midgut gland lipid content of *P. tasmanicus* ranged from 4.6% wet weight (19.1% dry weight) in B stage berried females to 14.7% wet weight (45.5% dry weight) in C stage non-berried females, and from 10.2% wet weight (29.7% dry weight) in B stage males up to 21.5% wet weight (approximately 60% dry weight) in the D_2 stage male (see Tables 6.3, 6.4 and 6.9). This is comparable to the lipid content of the midgut glands of some decapod crustaceans (see Table 6.9).

Obviously, there are large variations in the midgut gland lipid values in decapods, with differences between species, between sexes, e.g. male and female *Palaemon carcinus*, and between different moult stages of the same animal, e.g. A and D_0 stage *Pachygrapsus marmaratus*. The lipid content of the midgut gland of *P. tasmanicus* does not differ markedly from the observed ranges of values given in Table 6.9, and the changes that occur during the year have already been considered. *Parastacoides tasmanicus* is unusual in one respect. Some of the decapods mentioned in the table such as *Panulirus polyphagus*, *Palaemon carcinus* and *Carcinus maenas* become inactive during winter months (George and Patel, 1954; Heath and Barnes, 1970) and during this period their midgut gland lipid levels drop. *Parastacoides tasmanicus* also lowers its activity, or at least its oxygen

consumption, a great deal during winter, but the lipid levels in the midgut gland are maintained at pre-winter levels, or even increased.

Table 6.9 Lipid content of the midgut glands of some decapod crustaceans

Species*	Percentage lipid: Dry weight	Wet weight	Source
Palaemon carcinus ♂	5.4-59.9		George and Patel (1954)
" " ♀	32.6-44.3		" " "
Penaeus japonicus D_0		3.8	Ando *et al* (1977)
" " C_1-C_2		1.9	" " "
Birgus latro ♂	50-58		Lawrence (1970)
Birgus latro	74±9.3		Chakravarti and Eisler (1961)
Coenobita perlatus	54±3.4 (7)		Lawrence (1976a)
C. brevimanus	57±6 (9)		" "
C. rugosus	56±1.8 (6)		" "
Cancer antennarius	8.8		Giese (1966)
C. magister	17.5±3.6		" "
C. pagurus	29.6		Renaud (1949)
Carcinus maenas†		6.3±0.4	Chappelle (1977)
Eriocheir sinensis†		9.3±0.8	" "
Metapenaeus affinis ♀	10.6-35.8		Pillay and Nair (1973)
Portunus pelagicus ♀	15.6-39.2		" " " "
Pachygrapsus marmoratus A	11.9		Lautier and Lagarrigue (1976)
" " D_0	42.4		" " " "
Uca annulipes ♂	16.1-38.4		Pillay and Nair (1973)
" " ♀	15.6-36.4		" " " "
Panulirus polyphagus ♂	10.7-27.0		George and Patel (1954)
" " ♀	12.9-30.6		" " " "
Homarus vulgaris†		10.8±1	Chapelle (1977)
Orconectes limosus	?-70		Collatz (1969)
O. nais	25-75		Armitage *et al* (1972)
Parastacoides tasmanicus ♀	19.1-45.5	4.6-14.7	This study
" " ♂	29.7-60	10.2-21.5	

* Including sex or moult stage in some cases
† Starved for two weeks prior to test
N.B. Some of these ranges may not be the absolute range as animals may be unavailable at some times of the year, e.g. The values for female *P. tasmanicus* do not cover the entire range of values as females in the D - A moult stages were unavailable.

Apart from lipid, the glycogen and protein content of the midgut gland of *P. tasmanicus* can also be compared with that of other crustaceans. Glycogen levels in female *P. tasmanicus* range from 0.83% wet weight to 1.12% (2.9 - 4.7% dry weight) while in males the range is 0.44% to 1.11% wet weight (2.1 - 3.6% dry weight) (see Tables 6.3 and 6.4). The dry weight glycogen content of the midgut glands of 6 species of decapods ranged from 0.4% to 7% (Giese, 1966; Pillay and Nair, 1973; Ramamurthi and Veerabhadrachari, 1975; Zandee, 1966) while the total carbohydrates of the midgut glands of 3 species of decapods was measured at 0.6 - 4.6% dry weight (Lawrence, 1976a). The freshwater crayfish, *Orconectes virilis*, has a mean carbohydrate content of 1.80% wet weight (Hazlett *et al*, 1975). Thus it would appear that most of the carbohydrate in the midgut glands of decapods is in the form of glycogen, and that the glycogen levels of *P. tasmanicus* are unexceptional.

The protein content of the midgut glands of female *P. tasmanicus* ranges from 7.4% to 8.3% wet weight (20.4 - 34.6% dry weight) while for males the range is 4.5% to 9.2% (15.3 - 29.5% dry weight) (see Tables 6.3 and 6.4). Once again these values are within typical ranges. The protein content of the midgut glands of 7 species of decapods ranged from 6% to 43.1% dry weight (Giese, 1966; Lawrence, 1976a; Pillay and Nair, 1973) while 3 other species had wet weight values of 5.0% to 8.0% (Cannon and Grant, 1970; Hazlett *et al*, 1975).

As stated in Section 6.1, the eggs of marine invertebrates exhibit a wide range in the size of their energy stores, which are normally in the form of lipids. Fresh *P. tasmanicus* eggs have 21.7% wet weight (51.0% dry weight) lipid. This corresponds very closely to the 28.0% wet weight (51.9% dry weight) lipid in the ovaries of C stage non-berried females. These lipid levels in the eggs and ovaries are fairly high compared to other decapods. Table 6.10

gives the lipid levels of the eggs and/or ovaries of some decapod crustaceans.

Table 6.10 Lipid content of the eggs and/or ovaries of some decapod crustaceans

Species	Tissue	Lipid*	Source
Palaemon carcinus	ovaries	33.7-36.3	George and Patel (1954)
Eupagurus bernhardus	eggs	29.5	Pandian and Schumann (1967)
Barytelphusa cunicularis	ovaries (spent)	12.5	Diwan and Nagabhushnam (1974)
" "	ovaries (ripe)	37.5	
Cancer antennarius	ovaries	26.0	Giese (1966)
Metapenaeus affinis	ovaries (spent)	10.5	Pillay and Nair (1973)
" "	ovaries (ripe)	27.4	
Pachygrapsus marmoratus C_4	ovaries	30.8#	Lautier and Lagarrigue (1976)
Portunus pelagicus	ovaries (spent)	4.9	Pillay and Nair (1973)
" "	ovaries (ripe)	10.8	
Uca annulipes	ovaries	8.6-19.7	" " " " "
Panulirus polyphagus	ovaries	8.3-27.1	George and Patel (1954)
Orconectes nais	ovaries (ripe)	30	Armitage *et al* (1972)
Parastacoides tasmanicus	ovaries(spent)†	36.8	This study
" "	ovaries (ripe)	51.9	" "
" "	eggs	51.0	" "

* Percent dry weight.
\# This level rises considerably during moult but drops again afterwards.
† From C stage berried females. The lipid level in ovaries of B stage berried females is possibly lower but cannot be measured.

The fresh eggs and ripe ovaries of *P. tasmanicus* contain a larger lipid reserve than the eggs and/or ovaries of many other decapods. This is probably because *P. tasmanicus* carries the eggs and developing young for 8 months or more before they become independent and start feeding, and therefore they (the young) need a large lipid store to sustain them for this length of time. The wet

weight lipid level of juveniles that have just left their mother is 7.65% (see Table 6.5), or 35.9% lipid on a dry weight basis.

In addition to producing eggs with a large lipid store, *P. tasmanicus* also produces a relatively small number of 'large' eggs, when compared to many marine decapods. For example, the freshwater crab, *Eriocheir* sp., can spawn 920 000 ova at once (Waterman and Chace, 1960) whereas *P. tasmanicus* produces about 35 - 80. Other freshwater crayfish produce a small number of eggs as well, when compared to marine crabs. *Astacus* sp. produces 50 - 150 (Waterman and Chace, 1960) and *Orconectes immunis* produces up to 290 (Tack, 1941), while other members of the genus *Orconectes* produce a maximum of from 160 to 1015 (Momot, Gowing and Jones, 1978). *Procambarus clarkii* and *Cambarus bartoni* produce up to 652 eggs and 133 eggs respectively, while the very small *Cambarellus shufeldtii* produces a maximum of 60 (Crocker and Barr, 1968). Absolute weights of eggs are hard to find, but it would seem that the eggs of *P. tasmanicus* are likely to be large even among the freshwater crayfish, as species of comparable size produce far more eggs. For example, female *Orconectes immunis* with a cephalothorax length of 30 mm produce approximately 180 eggs (Tack, 1941) whereas a *P. tasmanicus* female of the same size would have about 55 (Lake and Newcombe, 1975). Another Tasmanian burrowing crayfish, *Engaeus cisternarius*, also produces relatively small numbers of eggs. Suter (1975) found that a specimen of *E. cisternarius* with a carapace length of 30.7 mm was carrying 67 eggs. However, the small number of eggs carried by this species may be partially attributed to the fact that this animal has a very short abdomen, as *E. fossor*, a closely related species with a longer abdomen carries more eggs. An animal of this species with a carapace length of 28.2 mm had 104 eggs. This is still a smaller number of eggs than is carried by

non-burrowing crayfish, and it is possible that production of a small number of large eggs is an adaptation to a burrowing existence.

Although the lipid levels in the ovaries and fresh eggs of *P. tasmanicus* are high (on a dry weight basis) the protein levels are fairly low (see Table 6.3). The values of 13.9% dry weight (5.9% wet weight) and 13.0% dry weight (7.0% wet weight) protein in fresh eggs and mature ovaries respectively, are low compared to the values in the ovaries and eggs of other decapod crustaceans (see Table 6.11). Of course one of the reasons why the protein levels appear to be so low is that the lipid content of the eggs and ovaries is so high. The glycogen content of ripe ovaries and fresh eggs of *P. tasmanicus*, 1.5% and 1.9% dry weight (0.83% and 0.82% wet weight) respectively, are similarly unremarkable, and quite close to the calculated values for the freshwater crayfish, *Orconectes nais*, although on a lipid-free dry weight basis the glycogen content of *P. tasmanicus* ovaries and eggs is considerably higher than that of *O. nais* ovaries (3.1% and 3.9% compared to 2%, respectively). Obviously, if lipid levels in the two species were comparable *P. tasmanicus* would have a much larger glycogen store in eggs and ovaries than *O. nais*. Glycogen would appear to play only a minor role as an energy store in ovaries and eggs of Crustacea as it is present in low amounts in most cases.

Table 6.11 Protein and glycogen content of ripe ovaries and eggs of some decapod crustaceans.

Species	Tissue	Protein*	Glycogen*	Source
Barytelphusa cunicularis	ovaries	46	0.75	Diwan and Nagabhushnam (1974)
Cancer antennarius	ovaries	59.3	0.5	Giese (1966)
Eupagurus bernhardus	eggs	66.6	-	Pandian and Schumann (1967)
Metapenaeus affinis	ovaries	39.0	1.3	Pillay and Nair (1973)
Portunus pelagicus	ovaries	52.1	3.3	
Orconectes nais	ovaries	-	1.5#	Armitage *et al* (1972)
Parastacoides tasmanicus	ovaries	13.0	1.5	This study
" "	eggs	13.9	1.9	" "

* Percent dry weight

\# Given in source as about 2% lipid-free dry weight.

The composition of the chelae and abdominal muscles of *P. tasmanicus* males and females does not vary as much as the composition of the other tissues discussed (see Tables 6.3 and 6.4). Lipid content varies between about 3% and 7% dry weight (0.60 - 1.34% wet weight), while glycogen varies between about 0.2% and 7.5% dry weight (0.032 - 1.47% wet weight), and protein varies from about 25% to 55% dry weight (4.35 - 10.10% wet weight). Much of this variation is due to changes associated with the stages of the moult cycle, as already discussed. If only the B and C stages are considered, the glycogen, lipid and protein ranges are 1.7 - 5.7% dry weight (0.45 - 1.14% wet weight), 3.5 - 7.1% dry weight (0.74 - 1.30% wet weight) and 29.2 - 39.8% dry weight (6.40 - 9.43% wet weight) respectively. There are some slight differences between the chelae and abdominal muscles, and between males and females, and this accounts for some more of the variation. The values are all comparable to the glycogen, lipid and protein contents of various other decapods as reported by Ando *et al.* (1975), Chapelle (1977), Hazlett *et al* (1975), Lawrence (1970), Pillay and Nair (1973) and Spindler-Barth (1976).

Juvenile *P. tasmanicus*, with the exclusion of those that have just left their mother, and the single animal available in the D_1''' - D_2 stage of the moult cycle, have glycogen, lipid and protein levels of 3.5% to 4.0% dry weight (1.04 - 1.16% wet weight), 4.8% to 7.3% dry weight (1.44 - 2.10% wet weight) and 19.8% to 20.4% dry weight (5.85 - 5.90% wet weight) respectively (see Table 6.5). The glycogen content is similar to the carbohydrate content reported for some marine sergestid shrimps by Donaldson (1976) but is considerably less than the carbohydrate content of 9 species of freshwater cladocerans and copepods tested by Vijverberg and Frank (1976). The lipid content of these juvenile *P. tasmanicus* is much

less than that of 5 species of estuarine crustaceans tested by Moore (1976), the copepods and cladocerans examined by Vijverberg and Frank (1976) and the sergestid shrimps examined by Donaldson (1976), but is similar to that of 8 species of planktonic crustaceans tested by Takahashi and Yamada (1976), *Palaemon paucidens* (Teshima and Kanazawa, 1976), *Pleuroncodes planipes* (Smith *et al*, 1975) and *Orconectes rusticus* (Wolfe, Rao and Cornwell, 1965). The protein content is also less than that of the copepods, cladocerans and sergestid shrimps but slightly higher than that of *Pleuroncodes planipes* on a wet weight basis, and similar to this latter species on a dry weight basis.

Parastacoides tasmanicus can be compared with several of the *Orconectes* species of freshwater crayfish from North America with respect to some specific characteristics. Thus the chitin content of *Orconectes propinquus* was measured by Stein and Murphy (1976); these authors obtained values similar to those reported here for *P. tasmanicus* (on a dry weight basis). The water content is also similar in the two species (Stein and Murphy did not remove the 'water of hydration' but their 'dry weight' values are similar to those of *P. tasmanicus*), as is the 'percent inorganic content' (percent ash), except for post-moult crayfish, where the inorganic content of *O. propinquus* is less than that of *P. tasmanicus*.

Another of the *Orconectes* species, namely *O. nais* (and probably others) has a life cycle that is in some ways similar to that of *P. tasmanicus*, but in other respects is very different. Like *P. tasmanicus*, *O. nais* also has an active period during the warm part of the year, and a period of quiescence or lesser activity during the colder months (Armitage *et al*, 1972). With *O. nais*, oviposition occurs in the spring, and while they are carrying the eggs and young the females build up energy reserves

so that after the young have left they can carry out a late spring moult, or even two moults (Armitage *et al*, 1973). After these moults vitellogenesis is stimulated when daylengths shorten, and there is no further moulting (Armitage *et al*, 1973; Rice and Armitage, 1974a). In autumn the animals enter burrows, where they remain until spring, and while the females are in these their ovaries finish developing, and remain in that state until early spring, when oviposition occurs again (if they live that long) (Rice and Armitage, 1974a). In *O. nais* preparations for moulting and vitellogenesis are mutually exclusive, but both are fitted into the 'short year'. In contrast, female *P. tasmanicus* accumulates energy reserves for the moult, in which there is an increase in size, and for growth of the ovaries, simultaneously. However, *P. tasmanicus* takes two years to do what *O. nais* does in one. This could be due to one or both of the following:

(1) The low metabolic rate of *P. tasmanicus* combined with a lower mean summer temperature than occurs in the habitat of *O. nais*;

(2) The long period that *P. tasmanicus* carries the eggs and young may make it impossible to separate moulting and oviposition by more than a few months, which would place too great a strain on the female's energy stores, especially as she cannot build up energy stores during winter or early summer, due to her inactivity.

The solution is apparently to build up both the ovaries and energy stores during the end of one summer, winter and the beginning of the next summer, after a moult in which there is no size increase, so that the female can moult and still be ready to produce and carry eggs for the next season. The difference between female *O. nais* and *P. tasmanicus* is reflected in a difference between the males of the species. Male *O. nais* moult (at least) twice yearly (Armitage *et al*, 1973) while adult *P. tasmanicus* males moult only once.

Most measurements of energy values of aquatic invertebrates fall within the range found by Prus (1970) for a wide range of such animals, namely between 17·58 and 28·46 kJ/g ash-free dry weight. Examples of studies which obtained values that agreed with this range are Moshiri and Cummins (1969), Laurence (1976), Wissing *et al* (1973), and Wissing and Hasler (1971). The highest values have usually been found amongst planktonic animals (mainly Crustacea) with large lipid stores that enable them to survive over winter. Griffiths (1977) gave values of 21·30 kJ/g ash-free dry weight for benthic malacostracans and 25·50 - 31·80 kJ/g ash-free dry weight for pelagic malacostracans. He stated that the difference in energy values between the two groups is attributable to a high lipid content in the high group, in support of Slobodkin and Richman's (1961) hypothesis that organisms should have low energy contents unless they are liable to a period of food scarcity during their life cycle.

Energy values for *P. tasmanicus* tissues, and for juveniles (see Tables 6.6 and 6.7) fall close to the mean of Prus's range of values, namely 23·40 kJ/g ash-free dry weight, with higher values being found mainly in the gonads and the eggs, and to a lesser extent the midgut glands; all these tissues have high lipid concentrations. The 'remainder' values (21·28 - 22·52 kJ/g ash-free dry weight (mean values)) are slightly lower than the other tissue values, but are higher than values quoted for crabs by Golley (1961) of 18·40 kJ/g ash-free dry weight. When the total energy content of *P. tasmanicus* is calculated it is found to be slightly higher than the values found by Griffiths (1977) for benthic malacostracans (21·30 kJ/g ash-free dry weight), but it is considerably less than the values

given by this author for pelagic malacostracans (25·50 - 31·80 kJ/g ash-free dry weight).

As stated above, the energy content of the 'remainder' which is 80 - 90% of the total dry weight of *P. tasmanicus* seems to be higher than *whole body* energy values of many crabs and crayfish. For examples, the freshwater crayfish, *Cambarus robustus* (Prus, 1970) and *Cherax destructor* (Woodland, 1967), have mean energy contents of 18·85 kJ/g ash-free dry weight and 21·08 kJ/g ash-free dry weight, respectively, while values for *P. tasmanicus* 'remainder' are above 21·28 kJ/g ash-free dry weight. In many cases the low values, in terms of J/g ash-free dry weight, of crustaceans which have moderately to heavily calcified exoskeletons are due, not to inherently low values in the animals concerned but rather to an underestimation of the ash content of the animals, e.g. Golley (1961), or to a failure to make allowances for water of hydration in the exoskeleton, e.g. Golley (1961), Rodgers and Quadri (1977), Salonen *et al*, (1976), Wissing and Hasler (1971), Wissing *et al* (1973) and Woodland (1967). All of these authors were partly concerned with animals with a moderate to large percentage of ash, yet none of them measured 'water of hydration'. Although in some cases it was not possible to obtain the original papers from which Prus (1970) or Griffiths (1977) obtained their data, it is considered unlikely that the 'water of hydration' would have been measured in these either. Paine (1971) discusses many of the possible sources of error in measuring the energy content of substances with a high inorganic content, and goes so far as to state "I believe a healthy scepticism should be maintained towards all caloric values determined from materials with ash contents in excess of 25% until the thermochemical consequences have been properly evaluated." With this in mind it *appears* that organic materials in the

exoskeleton of *P. tasmanicus* has a similar energy content to organic material in other tissues.

As was stated earlier, energy values are often of little use as indices of nutrition without associated measurements of the water content of the relevant tissues. A good example can be given from the results obtained here. The energy content of gonads of C stage berried females was 28 63 ±023 (4)kJ/g ash-free dry weight, while the energy content of gonads of C stage non-berried females was 30 14 ±079 (6)kJ/g ash-free dry weight. There is no statistically significant difference between these two values. However, the water content of the gonads of the berried females was 80.0 ± 3.2% while in the gonads of the non-berried C stage females it was only 58.5 ± 3.7%. On a wet weight basis the latter thus have more than twice the energy content of the former.

To summarise, the composition of the tissues of *P. tasmanicus* does not differ markedly from the tissues of other decapod crustaceans, apart from the fact that *P. tasmanicus* has eggs and gonads with a high lipid content. The energy content of the whole body is also similar to that found for benthic malacostracans, and any differences may generally be attributed to incorrect techniques used by other workers. However, *P. tasmanicus* does differ from other decapods in a number of ways. Firstly, it has a very low metabolic rate for its size, and this varies considerably with the season. Some of the advantages of this low metabolic rate will be seen in Chapter 7. Associated with this is the very unusual (for a freshwater crayfish) method of breeding that has been adopted by *P. tasmanicus*, and the way that metabolism, moulting and breeding interact is very interesting.

The growth and reproductive strategy of *P. tasmanicus*, involving as it does rapid growth during summer with almost no growth during winter due to the low metabolic rate, and with females breeding only

once every two years, may not at first sight appear to be an appropriate strategy, but it obviously is very successful. *P. tasmanicus* has no crayfish competitors on the button grass plains through most of its range, and there are no known predators of crayfish in burrows, so the only requirement, as far as breeding is concerned, is to produce enough young each year to inhabit the burrows that become available due to the death of the previous occupants. Successful juveniles will be those that are a large size when they leave their mother, and that then grow rapidly *during their first summer*, so that if they find an empty burrow, they can occupy it and defend it against other juveniles. By the end of the summer-mid-autumn period, competition for burrows would be much reduced, as juveniles that were unable to find an unoccupied burrow (or to dig one themselves) would have died due to predation or dehydration during the periods when the surface water dried up. By June-July, first year crayfish only make up about 15% of the population (see Chapter 2) so the death rate of adult crayfish is fairly low, suggesting that adults may be reasonably long lived.

Successful females, in terms of the reproductive strategy outlined above, would be those that lived the longest and in doing so produced the maximum number of large, rapidly-growing juveniles, with the emphasis on the size rather than the number of juveniles. Berried females would be best served by remaining quietly in their burrows through the whole of the egg-carrying period, thereby ensuring a minimum loss of eggs. This, coupled with the low metabolic rate and the short period in which to accumulate energy reserves before the next breeding season, means that these females would be unable to moult 'properly' in the following year and produce another batch of eggs. However, the low metabolic rate,

and the fact that these animals only incur the stress of reproduction every second year, might mean that these animals live for a considerable time, say 7 or 8 years or longer. This was the life span suggested by Newcombe (1970), but as stated in Chapter 2, he had no real basis on which to form this conclusion. It could be reasoned that if the recruitment of juveniles is only 15% per year then females at least would have to live 7 years, with only minor mortality in the 1 - 7 year age classes, if the population is to remain stable. This strategy is very different to that of the members of the genus *Orconectes*, which grow rapidly, breed each year but only live for 2 to 4 years, and is also very different from those crayfish that breed nearly continuously, such as *Astacus astacus*, *Paranephrops planifrons* (data in Momot *et al*, 1978, from a number of sources) and *Cherax destructor* in suitable conditions (B. Mills pers. comm.). Each method undoubtedly has advantages in the geographical and environmental conditions that reign in the regions where the various species occur.

7. RESPONSES of *Parastacoides tasmanicus* TO LOW OXYGEN LEVELS

7.1 Introduction

Beadle (1961) wrote "The primary condition for oxygen depletion in natural waters is an abundance of organic matter, usually of vegetable origin, providing substrates for the metabolism of oxygen-consuming micro-organisms. The process is, of course, accelerated by a rise of temperature, by the reduction of water movements which would favour the entry of atmospheric oxygen, and by the inhibition of photosynthesis through shading from light.... Such conditions are to be found in waterlogged soils, especially those of high organic content.... and in stagnant marshes and swamps." The button grass plains, on which *Parastacoides tasmanicus* lives, qualify admirably as areas in which oxygen-depleted waters would be expected to be found, and as was shown in Chapter 2, this is in fact the case. Oxygen levels as low as 0.8 mL/L have been measured in the water in crayfish burrows on button grass plains, and lower levels could be expected to to occur during warm, dry weather, when there would be high micro-organism, plant and animal metabolism, and little movement of sub-surface water. It would therefore be expected that *P. tasmanicus* would have some mechanism(s) for coping with low-oxygen conditions.

Crustaceans and other aquatic invertebrates can show a number of ways to cope with low-oxygen conditions. Firstly, and obviously, they may be able to detect changes in water oxygenation, and avoid poorly oxygenated areas. For example, some intertidal amphipods leave clumps of seaweed during low tide when the water in the seaweed becomes deoxygenated (Wieser and Kanwisher, 1959). *Gammarus oceanicus* actively avoids regions of anoxia in a preference chamber (Cook and Boyd, 1965), while adult *G. pulex*

detect and avoid dissolved oxygen tensions even well above those which may be lethal. When they are given the choice in a preference chamber, they show a preference for fully oxygenated water over water with 5.1 mL O_2/L or less. Young *Gammarus*, however, are indifferent to water with oxygen levels above 2.9 mg/L, and all animals are more tolerant of low oxygen levels at low temperatures (Costa, 1967). The freshwater crayfish, *Astacus astacus* also responds to an oxygen gradient by moving from areas with low oxygen levels to better oxygenated areas (Hoglund, 1961).

Some animals escape from poorly oxygenated waters by leaving the water and respiring in air. At low oxygen tensions, *Carcinus maenus* attempts to raise the anterior portion of its body above the surface of the water, and at 17°C it emerges from sea water at an oxygen concentration of 2.03 mL/L, while at 6°C it does not emerge until the oxygen level falls to 0.78 mL/L (Taylor and Butler, 1973; Taylor, Butler and Sherlock, 1973; Uglow, 1973). Similarly, *Orconectes immunis* may be seen at the margins of ponds and on emergent vegetation, with its carapace and gills exposed to the air, during periods of low oxygen tension in the water (Bovbjerg, 1970). However, this avoidance response is not always an appropriate way for animals to escape from poorly oxygenated areas. For example, a stream crayfish, *Orconectes propinquus*, which left the water of a river during a period of low oxygen tension, died on the bank (Jewell, 1920), presumably from the combined effects of suffocation, dehydration and heat exhaustion.

In contrast to the many animals that actively avoid poorly oxygenated waters, there are some animals which are normally resident in low oxygen environments, such as the amphipod, *Corophium volutator* (Gamble, 1971), that are indifferent to the oxygen tension, or which even prefer deoxygentated waters under certain conditions.

If an animal cannot, or will not escape from water with a low oxygen tension, then it has to make do with the oxygen that is available. Many animals are able to satisfy their oxygen requirements even when they are in poorly oxygenated waters, and some animals can maintain a constant uptake from normoxic to almost anoxic conditions. For example, the crabs, *Uca pugnax* and *U. pugilator*, are able to maintain a constant oxygen uptake in water with as little as 0.3 - 0.4 mL O_2/L at 20°C (Teal and Carey, 1967). Below this 'incipient limiting tension' oxygen consumption is proportional to oxygen tension. Davis (1975) defined the incipient limiting tension (ILiT) as "the point at which metabolic rate ceases to be dependent on available oxygen", when increasing oxygen tensions are being referred to, which is the same as the point at which metabolic rate ceases to be independent of available oxygen, when decreasing oxygen tensions are referred to. The burrowing shrimp, *Callianassa californiensis* (Crustacea:Thalassinidea), can also maintain a constant oxygen uptake down to very low oxygen levels, and has an ILiT of 0.4 - 0.8 mL O_2/L at 10°C (Pritchard and Kasparek, 1966; Thompson and Pritchard, 1969).

Different animals regulate their oxygen consumption down to different oxygen tensions (Mangum and van Winkle, 1973; van Winkle and Mangum, 1975; see for examples Ansell, 1973; Batterton and Cameron, 1978; Hiestand, 1931; Johansen *et al*, 1970; Jouve and Truchot, 1978; McLeese and Watson, 1968; McMahon and Wilkens, 1975; Miller, Pritchard and Rutledge, 1976; Nimura and Inoue, 1969; Spaargaren, 1977; Taylor, 1976b; Teal and Carey, 1967; Wiens and Armitage, 1961; Young, 1973) and some animals in fact have oxygen consumptions that are related to oxygen tensions over the entire range of oxygen tensions (Davis, 1975; Mangum and van Winkle, 1973; van Winkle and Mangum, 1975; see for examples Coyer, 1977; Hiestand, 1931; Leffler, 1973; Moshiri *et al*, 1970; Thomas, 1954 (see below)).

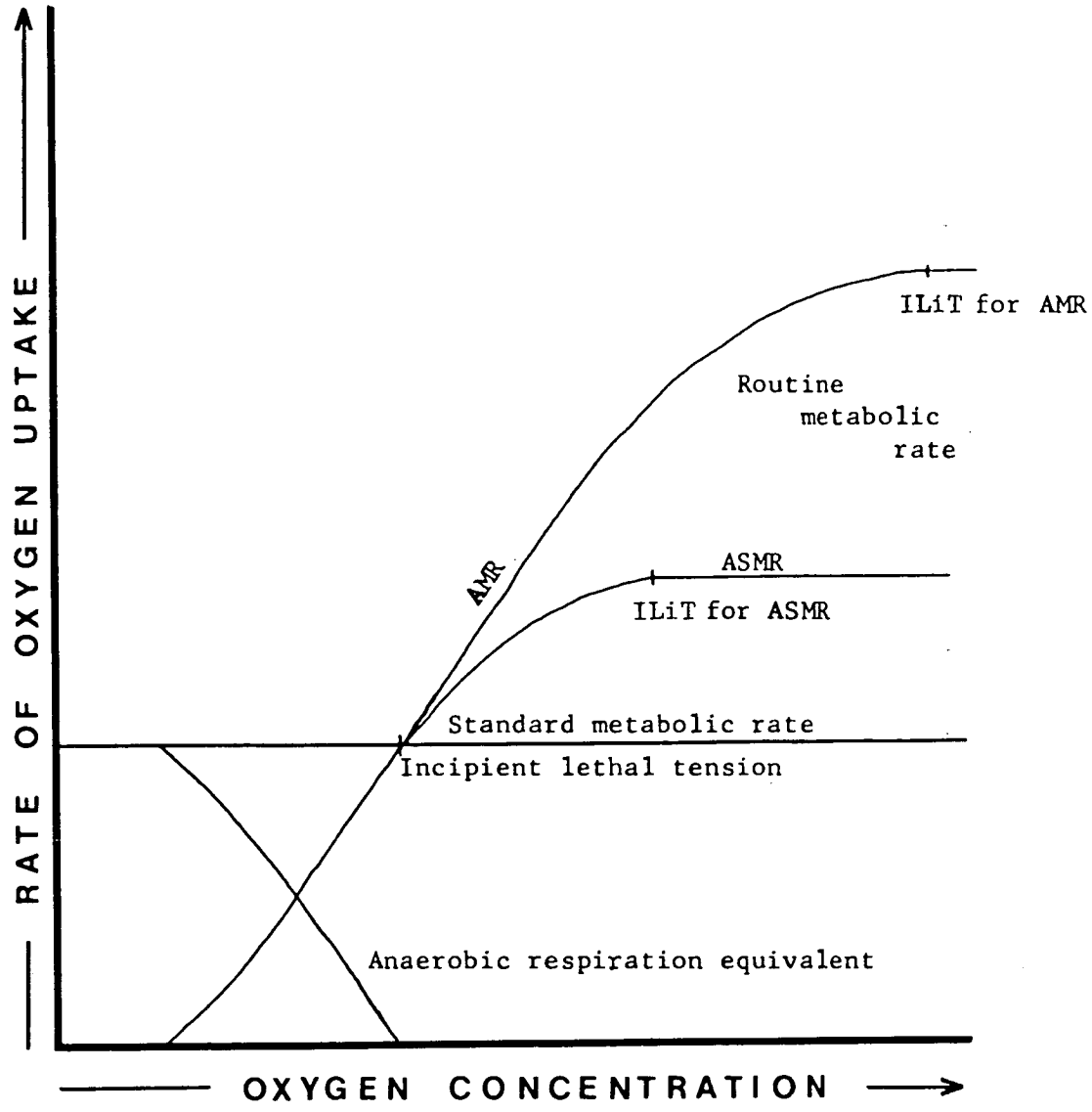
The classification of animals into 'oxy-regulators' and 'oxyconformers' is mainly an artificial one, and the same animal can sometimes act in either capacity, depending on the circumstances. The lobster, *Homarus gammarus*, can regulate oxygen uptake from water containing 1.4 - 4.9 mL O_2/L during spontaneous (routine) activity (Spoek, 1974), and therefore it can be classed as a regulator. However, Thomas (1954) found that restrained *H. gammarus* had an oxygen consumption that was proportional to the oxygen tension. The probable explanation for this is that the restrained lobsters were agitated, and oxygen consumption measurements were actually of 'active rate'; under these conditions the lobsters were oxyconformers.

The entire range of responses of oxygen consumption to oxygen tension can be shown with the aid of a diagram (see Figure 7.1, originally from Hoar (1966) but modified with ideas from Nimura and Inoue (1969) and Teal and Carey (1967)). The diagram shows the relationship between oxygen tension and the rate of oxygen uptake of aquatic invertebrates, and applies to both 'oxyregulators' and 'oxyconformers'. For 'oxyregulators' the ILiT for the *apparent* standard metabolic rate* (ASMR) (the oxygen consumption at minimum activity levels), below which the oxygen consumption is related to the oxygen tension, is somewhere within the normal range of oxygen tensions encountered by animals, the better regulators having the lower ILiT. For 'oxyconformers' the ILiT for the ASMR is effectively higher than normal oxygen tensions encountered by the animals concerned.

*The *apparent* standard metabolic rate is different from the (real) standard metabolic rate, which is the minimum metabolic rate accompanying the energy cost of maintenance.

Figure 7.1 Relationship between rate of oxygen uptake (oxygen consumption) and external oxygen concentration. Abbreviations used here and in the text: active metabolic rate (AMR), apparent standard metabolic rate (ASMR), incipient limiting tension (ILiT). Original graph from Hoar (1966), but modified with ideas from Nimura and Inoue (1969) and Teal and Carey (1967). 'Routine metabolic rate' on the graph signifies oxygen consumption rates between the AMR and the ASMR.

Figure 7.1


RATE OF OXYGEN UPTAKE
ILiT for AMR
Routine
metabolic
rate
AMR
ASMR
ILiT for ASMR
Standard metabolic rate
Incipient lethal tension
Anaerobic respiration equivalent
OXYGEN CONCENTRATION

It can be seen how there can be confusion over whether an animal is an 'oxyconformer' or an 'oxyregulator', if by mistake the active metabolic rate (AMR) (the maximum possible metabolic rate under forced activity (Wycliffe and Job, 1977)), or a high routine metabolic rate (RMR) (spontaneous activity metabolic rate) is measured rather than the ASMR. The ILiT for the AMR (or high RMR) may be above normal oxygen tensions, and therefore the animal would be classed as an oxyconformer, whereas if the ASMR had been measured the animal would have been classed as an oxyregulator.

At oxygen tensions above the ILiT an 'oxyregulator' can maintain a constant ASMR despite varying oxygen tensions. Animals use various methods to achieve this. For example the rate of flow over the respiratory surfaces may be altered and/or a greater percentage of the oxygen in the water may be extracted as the oxygen tension is lowered. The shrimp, *Palaemon adspersus*, increases its scaphognathite beating rate over the range PO_2 150 - 40 mm Hg in an attempt to maintain its oxygen consumption rate at a steady level (Hagerman and Uglow, 1979). See Butler, Taylor and McMahon (1978), Childress (1971), Dejours and Beekenkamp (1977), McMahon and Wilkens (1977), Uglow (1973) and references for 'oxyregulators' for other examples.

Animals also increase the rate of flow of water over their respiratory surfaces, and/or increase the percentage of oxygen extracted from the water to obtain extra oxygen when they need to increase their activity. Obviously there is a limit to the amount of oxygen that an animal can transport to its tissues from the respiratory surfaces, and this places an upper limit on the AMR. Therefore, above a certain oxygen tension (the ILiT for the AMR) the AMR does not increase with increasing oxygen tensions.

As with the case of the ILiT of the ASMR for 'oxyconformers', this ILiT may be at an oxygen tension above the normal oxygen range. Below the ILiT for the AMR, the AMR is limited by the ability of the animal concerned to extract oxygen from the water i.e. by the maximum amount of water that can be pumped over the respiratory surfaces and the maximum amount of oxygen that the animal can extract from the water. As the oxygen tension is lowered, so the AMR is lowered.

If the oxygen tension is lowered far enough, the point is reached where the ASMR cannot be readily maintained (i.e. the ILiT for the ASMR is reached) and so the ASMR decreases as the oxygen tension decreases, although if necessary the animal can increase its (aerobic) activity to a limited extent. The animal lowers its ASMR by decreasing all non-essential activity. Many crustaceans reduce their heart rate at low oxygen tensions (Florey and Kriebel, 1974; Hagerman and Uglow, 1979; Hill and Koopowitz, 1975; Larimer, 1962; Spaargaren, 1977) and activity in general; for example *Homarus gammarus* (Spoek, 1974), *Macropipus holstatus* and *M. depurator* (Uglow, 1973) and the freshwater shrimp, *Caridina fernandoi* (Wycliffe and Job, 1977), become inactive at low oxygen tensions. At moderately low oxygen tensions an animal may increase certain of its respiratory activities to maintain its oxygen consumption, but at very low oxygen tensions it is either too energy-expensive to do this, or in fact the energy output is more than the oxygen-equivalent gain, and it is more practical to cut the respiratory activities and reduce the energy demand than to try to maintain the oxygen supply.

If the oxygen tension is lowered enough, eventually a point is reached where the available oxygen is only just sufficient to supply the minimum metabolic demands. The only way that any sort

of activity can be increased under these circumstances is if energy is supplied by anaerobic means. At this oxygen tension, called the 'incipient lethal tension', the *real* standard metabolic rate is reached, and AMR equals ASMR. At oxygen levels lower than this, continued survival depends on an energy source other than aerobic respiration to supply at least some of the energy required by an animal; for crustaceans and most other animals the survival time is limited.

The characteristics which distinguish animals adapted to living in low oxygen tensions from other animals are:

(1) A low ASMR, or more importantly a low RMR, such as *Parastacoides tasmanicus* has (see Chapter 6). This means that sufficient oxygen is available to satisfy oxygen demands for some time, even in small volumes of water where oxygen reserves would otherwise soon be depleted.

(2) An ILiT for ASMR that occurs at a low oxygen tension. This is important because it means that sufficient oxygen is available to satisfy normal demands even at low oxygen tensions.

(3) An incipient lethal tension that occurs at a low oxygen tension. This is the most important factor to consider with 'oxyconformers', as (1) and (2) do not apply. It is also probably the most important factor to consider with oxyregulators, since although animals are extremely restricted at oxygen tensions at, or just above, the incipient lethal tension, they are not, at this point, accumulating toxic byproducts of anaerobic respiration, and may consequently be able to survive for extremely long periods.

(4) An ability to utilise some of the oxygen present even at very low oxygen tensions, below the incipient lethal tension. This reduces the amount of energy that must be produced by anaerobic respiration, and thus prolongs survival time.

The two *Uca* species mentioned earlier (Teal and Carey, 1967) can reduce the oxygen tension in water down to 0.64 mm Hg (0.4% of an atmosphere), and this allows a small amount of aerobic energy production even at very low oxygen levels; Teal and Carey demonstrated that this option is not always available, since they found that *Sesarma cinereum* stopped its oxygen uptake at a higher oxygen tension, 3·19 mm Hg (approximately 2% of an atmosphere). Some animals are so efficient that they can deplete oxygen levels to exhaustion (Mangum, Kushins and Sassaman, 1970). Once again, the oxygen uptake at low oxygen levels is only useful if the extra oxygen obtained is in excess of that required to provide the extra energy expended (Beadle, 1961).

Anaerobic respiration in crustaceans is, as far as is presently known, restricted to the conversion of glycogen and/or glucose to lactic acid, with the associated production of a limited quantity of energy. The theoretical adenosine triphosphate (ATP) yield for the conversion of 1 mole of glucose to 2 moles of lactic acid is only equivalent to 83·7 kilojoules (20 kilocalories) whereas the conversion of 1 mole of glucose and 6 moles of oxygen to 6 moles of water and 6 moles of carbon dioxide has a theoretical yield equivalent to 1·59 megajoules (380 kilocalories) (Scheer, 1953). Not only is the relative energy production in anaerobic respiration low, but in addition the lactic acid produced is toxic, and if it is not excreted it will eventually reach lethal levels.

Lactic acid production, as a means of surviving in anoxic conditions is only useful to an animal if it subsequently returns to aerobic conditions, thus allowing it to remove the lactic acid, usually be reconverting it to pyruvate which can be aerobically metabolised, or if the animal can excrete the lactic acid as it produces it. Some crustaceans which produce lactic acid can

survive for reasonably long periods in anoxic conditions. For example, adult *Cyclops varicans* can survive 36 hours of anaerobiosis at 16°C (Chaston, 1969), and the burrowing shrimps, *Callianassa californiensis* and *Upogebia pugettensis*, survive three days anoxia at 10°C (Thompson and Pritchard, 1969). At 21°C *Uca pugnax* can survive at least 24 hours in anoxic conditions, and when it is returned to aerated conditions it 'pays back its oxygen debt' by increasing its oxygen consumption rate to above normal levels and utilising the lactic acid at about the same rate as it is produced in anoxic conditions (Teal and Carey, 1967). Even the shore crab, *Carcinus maenas*, is capable of surviving short exposures to anoxic conditions. Seven out of 12 animals survive after 20 hours exposure to anoxia at 6°C, but it took the survivors a long time in aerated water before they recovered (Spaargaren, 1977).

One of the problems with lactic acid is that it is likely to affect the acid-base balance of the body fluids. It is possible, however, that animals with a calcium carbonate skeleton or shell could neutralize the acid by forming calcium salts or calcium bicarbonate (Florey, 1966 and see below).

Of those animals that produce lactic acid in anoxic conditions, the ones that will survive the longest without oxygen will be;

(1) Those that have a low standard metabolic rate and therefore accumulate lactic acid slowly.

(2) Those that can tolerate the highest concentrations of lactic acid and/or can neutralize or excrete it.

Apart from anaerobic respiration involving lactic acid production, there are much more effective pathways that are used by many intertidal bivalves and other facultative anaerobes such as *Tubifex tubifex* (Schöttler and Schroff, 1976), *Arenicola marina* (Schroff and Schöttler, 1977) and *Chironomus thummi* Larvae (Wilps and Zebe, 1976). These pathways are efficient enough to free many of the organisms that use them of any ultimate requirement for

oxygen, although this is normally essential for animals which rely on lactic acid production (Hochachka, 1975). Carbohydrates are still the main source of energy, but fatty acids, protein and amino acids may also be mobilized. For example, in oyster muscle there is a 'stoichiometric coupling between anaerobic glucose and glutamate catabolism' (Hochachka, 1975). The end products can be one or more of acetate, alanine, butyric acid, caproic acid, carbon dioxide, glutamate, isobutyrate, isoualerate, lactate, malate, octopine, proline, propionate, pyruvate, succinate, and valeric acid and are frequently less toxic than lactic acid (see Bayne, *et al*, 1976; de Zwann, Mohamed and Geraerts, 1976; de Zwaan and Wijsman, 1976; de Zwaan and Zandee, 1972; Dries and Theede, 1976; Gade, 1975; Gade *et al*, 1975; Hochachka, 1975; Hochachka, Fields and Mustafa, 1973; Hochachka and Somero, 1973; Jones, 1972; Livingstone, 1978; Livingstone and Bayne, 1977; Storey, 1977; von Brand, 1946 for details of the various pathways). These end products are often excreted in which case the animal concerned can usually tolerate an indefinite exposure to anoxic conditions, but they are sometimes accumulated. If they are accumulated then there may be a repayment of the oxygen debt when the animal is returned to oxygenated conditions, as occurs with the bivalve, *Arctica islandica* (Taylor, 1976a). Accumulated end-products are likely to be buffered by dissolution of the shell in bivalves (Akberali, Marriott and Trueman, 1977; Dugal, 1939).

The animals using these methods of anaerobic respiration can usually survive for much longer in anaerobic conditions than do animals that accumulate lactic acid. For example, the nematode, *Eudorylaimus andrassyi*, and the oligochaete, *Euilyodrilus heustheri*, can survive for 6 months in anaerobic conditions at 15^{o}C by using these methods (Por and Masry, 1968).

The mechanisms discussed above are those that best allow an animal to survive in low oxygen or anoxic conditions.

Some animals do not respond in the ways mentioned, but the responses of these animals are usually such as to decrease their survival times rather than to increase them.

This investigation of the responses of *Parastacoides tasmanicus* to low oxygen and anoxic conditions will be discussed in sequence, as the reasons for carrying out some of the experiments are consequent on the results of the preceding test(s). Therefore the aim of each experiment will be made clear, followed by the method and results of the experiments. The results include a brief outline of what further experiments are indicated as being necessary; these further experiments are in turn reported in the same manner. This procedure enables the respiratory adaptations developed by *P. tasmanicus* in order to deal with an oxygen deficient habitat to be demonstrated in a logical and coherent manner.

7.2.1 Aim: To investigate behavioural responses of *Parastacoides tasmanicus* exposed to depleted-oxygen conditions.

7.2.2 Materials and methods

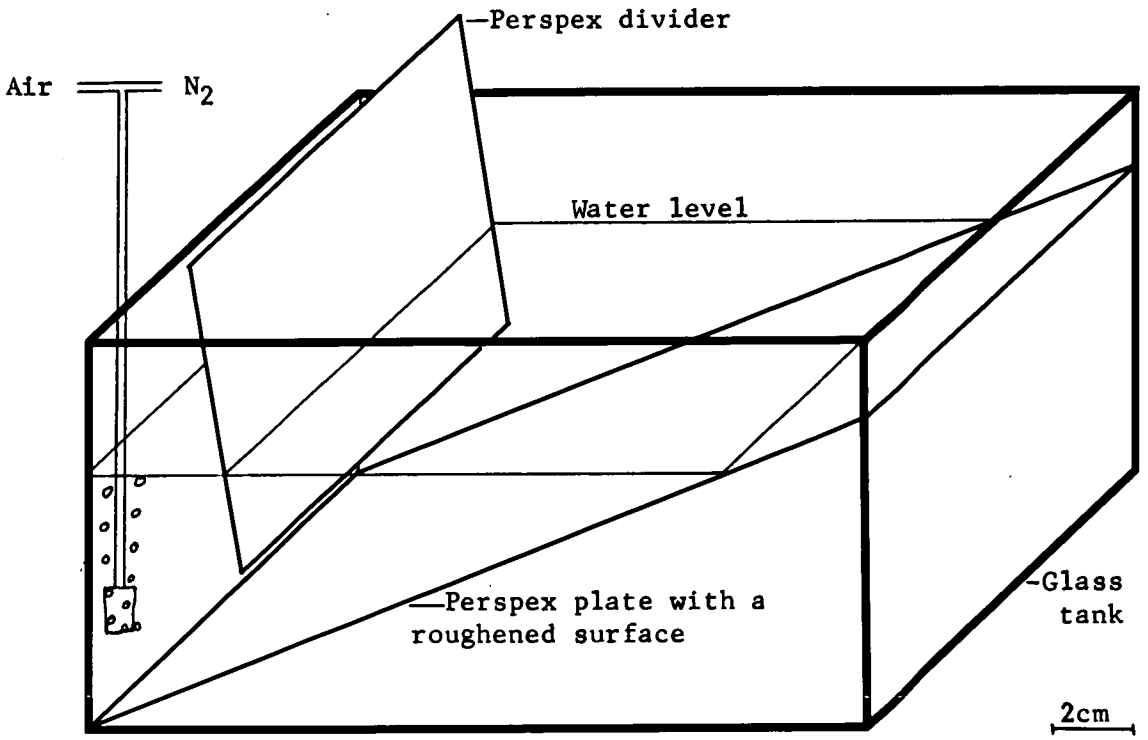
An animal was placed in the glass tank illustrated in Figure 7.2. This tank was placed in a large plastic container with a loose-fitting lid, which screened the animal from outside disturbance. Slits in the side of the container enabled the undisturbed animal to be observed. Air was bubbled through the air stone into the water in the tank for an hour while the animal settled down, after which the proportion of time that the animal spent totally in the water in the tank, and the proportion of time that it spent with at least its cephalothorax (and hence its gills) out of the water in the tank, was measured.

After a minimum observation period of 20 minutes the air supply was turned off, and high-purity nitrogen (Commonwealth Industrial Gases) was bubbled through the water in the tank. This rapidly reduced the oxygen content of the water to less than 1.5 mL/L and eventually reduced it to less than 0.8 mL/L. (In this section water with less than 1.5 mL O_2/L is referred to as being 'deoxygenated'). A slight air current was maintained through the large container to ensure that the gas over the water in the shallow end of the tank was of normal air composition.

The animal was observed to see if there was any change in the way that it apportioned its time between the air and the water. If any abrupt and distinct change occurred the 'response time' was noted. The percentage of time that the animal spent in and out of the water was again measured (after the response, if present), as well as the length of each period spent in or out of the water. Males and non-berried females of various weights were acclimated to and tested at, temperatures of 20^{o}, 17-18^{o}, 15^{o} and 5^{o}C. Unfortunately the number of animals available was too small to enable animals of the same sizes to be acclimated at each temperature. These animals were not starved or treated in any special way. In order

Figure 7.2 Apparatus used to investigate behavioural responses of *Parastacoides tasmanicus* in response to low oxygen levels.

Figure 7.2


Perspex divider
Air
N_2
Water level
Perspex plate with a roughened surface
Glass tank
2cm

to determine whether the response changed after prolonged exposure to low oxygen conditions, some animals were kept in deoxygenated conditions for extensive periods and the above observations repeated at intervals.

7.2.3 Results

The results of this experiment are given in Table 7.1. Apart from initial measurements of their time-partitioning in deoxygenated water, animals 5,6,18,20,8,10,11,12,13,14,15 and 16 were tested again at the times shown (in minutes), after the commencement of deoxygenation.

It is clear from Table 7.1 that at temperatures of 17°C and above most of the crayfish tested spent a significantly greater proportion of their time in the air when the water was deoxygenated than when it was oxygenated. At temperatures below 17°C,i.e. 15° and 5°C, the animals spent no more time in the air in deoxygenated conditions than in oxygenated conditions, and in fact prolonged exposure to low oxygen conditions at the lower temperatures resulted in animals spending significantly less of their time out of water (with the exception of animal number 10). Small animals tended to remain in the water at 17°C in both oxygenated and deoxygenated conditions, in contrast to the responses of the larger animals.

At all temperatures the normal pattern in oxygenated conditions was for the crayfish to spend 2 - 10 minutes in the water, then 'wander' out of the water for a short period before returning to the water and repeating the cycle. While out of the water the animals were quite mobile. The animals spent more time out of oxygenated water at low temperatures than they did at higher temperatures. This difference is statistically significant at a 5% level between 5° and 17 - 18°C and between 5° and 20°C.

Table 7.1 Partition of time between air and water by *Parastacoides tasmanicus* in oxygenated and deoxygentated conditions.

Animal number	Time+ (min)	Weight (g)	Temp* (°C)	Oxygenated conditions: Percent of total time spent in air	Oxygenated conditions: Time partitioning in minutes ‡ (Mean ± S.E. (N)) Air	Oxygenated conditions: Time partitioning in minutes ‡ (Mean ± S.E. (N)) Water	Deoxygenated conditions (oxygen concentration less than 1.5 mL/L): Time to response (min)	Deoxygenated conditions: Percent of total time spent in air after response	Deoxygenated conditions: Time partitioning in minutes ‡ (Mean ± S.E. (N)) Air	Deoxygenated conditions: Time partitioning in minutes ‡ (Mean ± S.E. (N)) Water
1		3.5	20	3.1	0.27± 0.04 (7)	7.35 ± 2.10 (8)	11.7	53.7	2.12 ± 0.32 (15)	1.97 ± 0.46 (14)
"		"	20	2.3	0.18± 0.04 (4)	5.86 ± 1.55 (5)	7.3	50.3	3.22 10.84 12.46	6.54 ± 2.60 (4)
2		1.4	20	1.4	0.25 0.29	10.03 12.80 15.63	19.0	36.2	3.48 4.17 4.86	2.50 20.00
3		3.4	20	9.2	0.46± 0.10 (4)	3.63 ± 0.98 (5)	4.0	75.7	2.97 0.32 (13)	0.95 0.14 (13)
4		3.0	18	2.4	0.08 0.48	4.01 6.45 7.03	17.3	69.4	4.62 ± 1.39 (8)	2.04 ± 0.47 (8)
5		7.6	17.5	6.1	0.31± 0.05 (6)	4.70 ± 1.37 (6)	19.4	69.1	3.73 ± 1.08 (4)	1.20 2.28 3.18
"	150-169	"	"				-	44.7	3.67 4.83	3.67 6.83
"	335-375	"	"				-	75.8	6.06 ± 2.02 (5)	1.62 ± 0.54 (6)
"	1126-1200	"	"				-	47.4	4.38 ± 0.87 (8)	4.86 ± 1.57 (8)
"	1201-1230	"	"	0.0	0.00	29.00 (1)				
6		2.3	17	3.1	0.50 0.68	9.68 12.17 14.99	20.4	80.4	10.80 ± 4.02 (7)	3.00 ± 0.32 (6)
"	360-423	"	"				-	66.7	4.65 ± 1.02 (9)	2.32 ± 0.33 (9)
7		0.1	17	0.0	0.00	20.00 (1)	No response	0.0	0.00	37.00 (1)
17		1.2	17	8.1	1.00 1.42	5.23 9.66 12.67	-	0.0	0.00	20.00 (1)
18		4.1	17	5.7	0.29 ± 0.03 (6)	4.04 ± 1.26 (7)	0.1	95.6	9.42 19.26	1.33 (1)
"	90-100	"	"				-	88.3	5.62 5.89 6.16	0.83 1.51
19		4.6	17	3.6	0.18 ± 0.01 (6)	4.13 ± 0.80 (7)	2.5	83.3	6.50 9.77 12.88	0.81 1.73 3.31
20		3.0	17	16.7	0.29 ± 0.01 (17)	1.39 ± 0.16 (18)	Uncertain	56.4	1.52 ± 0.08 (13)	1.17 ± 0.12 (13)
"	210-240	"	"				-	62.2	1.81 ± 0.30 (12)	1.20 ± 0.31 (11)
8		0.5	15	5.1	0.30 ± 0.16 (6)	4.74 ± 1.35 (7)	-	0.7	0.08 ± 0.01 (4)	10.55 ± 0.75 (4)
"	255-280	"	"				-	0.0	0.00	25.00 (1)
9		2.5	15	3.9	0.18 ± 0.03 (8)	3.95 ± 1.18 (9)	Uncertain	19.1	0.33 5.59	2.01 8.56 14.51
10		3.6	15	18.3	0.64 ± 0.16 (10)	2.86 ± 0.64 (10)	No response	16.2	0.89 ± 0.21 (6)	4.94 ± 1.18 (6)
"	125-180	"	"				-	34.1	2.83 ± 1.20 (6)	5.43 ± 3.25 (7)
11		0.5	15	9.8	0.33 0.48 0.66	3.39 ± 1.21 (4)	No response	10.3	0.97 ± 0.22 (5)	7.53 ± 3.57 (6)
"	783-843#	"	"				-	0.0	0.00	60.00 (1)
12		3.0	15	1.7	0.17 0.25	6.80 7.89 9.88	-	0.0	0.00	25.00 (1)
"	60-105	"	"				-	0.4	0.17 (1)	10.50 34.34
"	180-200	"	"				-	0.0	0.00	20.00 (1)
"	1440-1485	"	"				-	0.0	0.00	45.00 (1)
13		3.5	5	14.2	0.71 ± 0.17 (6)	3.68 ± 0.80 (7)	Uncertain	3.6	0.75 0.89	9.55 14.06 19.77
"	1080-1110	"	"				-	0.0	0.00	30.00 (1)
14		1.7	5	16.1	0.97 ± 0.07 (5)	4.20 ± 0.86 (6)	No response	10.2	0.96 1.24 1.40	7.86 ± 4.69 (4)
"	1320-1350	"	"				-	0.0	0.00	30.00 (1)
"	1680-1700	"	"				-	0.0	0.00	20.00 (1)
15		4.6	5	10.1	0.67 ± 0.06 (6)	5.07 ± 1.72 (7)	Uncertain	18.5	2.08 ± 0.53 (4)	7.33 ± 1.40 (5)
"	190-230	"	"				-	0.0	0.00	40.00 (1)
16		3.7	5	23.1	1.36 ± 0.45 (6)	4.48 ± 1.24 (6)	No response	29.4	2.02 ± 0.36 (8)	4.32 ± 1.55 (9)
"	115-135	"	"				-	0.0	0.00	20.00 (1)

\+ Time after the commencement of deoxygenation
* Acclimation temperature = test temperature
\# Lactic acid content at the end of this time was 0.336 mg/g wet weight.
‡ Length of periods spent in air and in water. N is the number of periods. Where N <4 the length of each period is given.

In deoxygenated conditions animals tested at the higher temperatures spent about 1 - 10 minutes in the water, and then moved up the ramp until their cephalothorax, but not their abdomen, was exposed to the air. They would lie motionless in this position for 1 - 15 minutes before moving back into the water for another 1 - 10 minute period. In contrast to their behaviour in oxygenated conditions they neither left the water completely nor did they move around, but they spent far longer with their cephalothorax out of the water. Even after as long as 19 hours in deoxygenated conditions (see results for animal number 5) the animals continued this pattern of partitioning their time between the air and the water.

At temperatures of 15°C and below, animals did not spend significantly longer with their cephalothorax out of water in deoxygenated conditions than in oxygenated conditions. There is no reason why the crayfish should not have left the water to replenish their oxygen supplies if they needed to do so when the temperature was 15°C or below. The fact that they did not do so in these experiments indicates that at temperatures of 15°C and below they could obtain enough oxygen to satisfy their requirements from the amount that was present (see Section 7.5 which shows how the animals reduce their oxygen requirements). This conclusion is supported by the fact that even after 14 hours in low oxygen conditions animal number 11 had a low total body lactic acid concentration of only 3·73 μmol/g wet weight (see Section 7.6). If lower oxygen levels could be obtained at the lower temperatures it would be expected that the crayfish would respond as they do at the higher temperatures.

One interesting point is that even though the cycle of an animal entering the water and leaving the water will continue for at least 20 hours at temperatures of 17°C and above, as soon as

the water becomes oxygenated again the response disappears, and the animal concerned starts to spend most of its time in the water again (see results for animal number 5 in Table 7.1).

Obviously *P. tasmanicus* leaves the water at high temperatures (and probably low temperatures) when the oxygen level is low enough. It was shown in Chapter 4 that crayfish can survive for long periods out of water, and therefore they must be able to respire in air to some degree. It would be useful to know whether or not aerial respiration is equivalent to aquatic respiration. If it is equally effective, then *P. tasmanicus* could still obtain enough oxygen to allow normal activity in 'dry' burrows, or in burrows in which only hypoxic water is available. As long as the humidity is high enough (see Chapter 4) the crayfish would not become dehydrated.

7.3.1 Aim: To compare the aerial and aquatic respiration of *Parastacoides tasmanicus*.

7.3.2 Materials and methods

The respiration of 12 adult *P. tasmanicus* in 200 mL of water was measured in 300 mL flasks on a Gilson respirometer at 15°C. As in previous experiments, all of the measurements were carried out at the same time of day, after the animals had been allowed to settle down in the vessels for 2 hours. The same *P. tasmanicus* also had their respiration in air measured at 15°C, either the day before or the day after the measurements in water. The measurements in air were carried out on the animals after they had been removed from water, placed in the empty Gilson vessels and allowed 2 hours to settle down. The humidity in the Gilson flasks was 100%, provided by a few drops of water.

7.3.3 Results

The results of the experiment are shown in Table 7.2.

Table 7.2 Aerial and aquatic respiration of *Parastacoides tasmanicus*.

Weight* (g)	Oxygen consumption* ($mL/g.h^{-1}$) Aerial	Aquatic
3.49 ± 0.48 (12)	0.041 ± 0.007 (12)	0.046 ± 0.008 (12)

* Mean ± S.E. (N)

There was no significant difference between the respiration rate of *P. tasmanicus* in water and the respiration rate of *P. tasmanicus* out of water at 15°C. This means that 'wet' crayfish respire normally and can remain active out of water, so long as the humidity is high. No tests were performed to see if the respiration rate is affected when crayfish start to dry out, but as suggested in Chapter 4, it is likely that the respiration rate would fall.

The respiration rates of crayfish reported above are higher than would be expected from the oxygen consumption measurements given in Chapter 6. There are two possible reasons for this. Firstly, the measurements here were made in a Gilson respirometer, and the movement of the flasks in this machine may have kept the animals in a higher activity state than if they were in undisturbed closed bottles. Secondly, the animals used here had all been kept in the laboratory for some time, and as stated previously (Chapter 6) this affects their respiration rates. However, neither of these possibilities would be expected to affect the relationship between aerial and aquatic respiration.

7.4.1 Aim: To determine the relationship between oxygen concentration and the oxygen consumption of *Parastacoides tasmanicus*.

As stated in Section 7.1, it is necessary to know the relationship between the oxygen concentration and the oxygen consumption of an animal if any inferences about its ability to survive in low oxygen conditions are to be made (see Figure 7.1). This relationship shows the range of oxygen concentrations over which an animal can regulate its oxygen consumption, and demonstrates how quickly oxygen consumption diminishes as the oxygen concentration is lowered below the incipient limiting tension.

7.4.2 Materials and methods

A number of animals weighing approximately 5 g each were placed into individual glass jars of 500 - 900 mL capacity. The jars were filled with button grass water which had been treated with 25 mg/L of neomycin and streptomycin, and then filtered, to inhibit bacterial growth. The jars were closed with perspex lids, each of which had a 5 mm hole in it, which was sealed with a rubber bung. The jars were kept in the dark at either 5^{o} or $15^{o}C$, in constant temperature rooms. At regular intervals the bungs were removed from the lids and 1 - 2 mL water samples were taken from the apertures. The water that was removed was replaced with water of approximately the same oxygen concentration and the bungs were then replaced. The oxygen content of the water samples was measured by the micro-Winkler technique (Fox and Wingfield, 1938). The oxygen consumption of the crayfish at various oxygen concentrations was calculated, and graphs of mean (± S.E.) oxygen consumption vs. oxygen concentration were constructed for crayfish at $5^{o}C$ and for crayfish at $15^{o}C$. The quadratic polynomials which best fitted the points on the graphs were calculated in accordance with the claims

of van Winkle and Mangum (1975) that the quadratic polynomial provides the best statistical fit for this sort of curve, although the semi-log equation shows almost as good a fit in some cases.

7.4.3 Results

Figures 7.3 and 7.4 illustrate the relationship between the oxygen concentration and the oxygen consumption of *P. tasmanicus* at 5° and 15°C respectively. The graphs show the mean (± S.E.) oxygen consumption at various oxygen concentrations and also show the polynomial curves that best fit the data. Table 7.3 supplies the actual values from which the graphs were constructed.

Table 7.3 Oxygen consumption of *Parastacoides tasmanicus* at different oxygen concentrations at 5° and 15°C.

5°C										
Oxygen concentration (mL/L)	0.2	0.4	0.8	1.2	1.6	2.2	3.0	3.8	4.6	5.4
Oxygen consumption (mL/g.h x 10^3)	0.30	1.27	4.06	6.58	8.22	10.63	13.30	13.11	12.58	15.14
S.E. (x 10^3)	0.28	0.33	0.48	0.84	0.93	1.05	1.35	1.88	1.60	1.70
N	5	15	15	15	14	13	12	11	9	7
15°C										
Oxygen concentration (mL/L)	0.2	0.4	0.8	1.2	1.6	2.2	3.0	3.8	4.6	5.4
Oxygen consumption (mL/g.h x 10^3)	2.00	7.18	9.44	14.37	16.03	17.36	18.70	22.03	22.88	23.07
S.E. (x 10^3)	0.58	1.59	1.75	1.99	2.12	2.04	2.65	3.15	3.01	2.61
N	5	8	9	10	10	10	10	10	10	10

The polynomial equations of the form $y = B_0 + B_1x - B_2x^2$, where y is the oxygen consumption in mL/g/h and x is the oxygen concentration in mL/L, with B_0, B_1 and B_2 empirically determined constants, are shown in Table 7.4.

Figure 7.3 Relationship between oxygen concentration and oxygen consumption of *Parastacoides tasmanicus*, at 5°C (Mean ± S.E., see Table 7.3 for N values). The curve is the quadratic polynomial which best fits the points, but this equation is only valid to the point of inflexion. Above this point the oxygen consumption is independent of oxygen concentration.

Figure 7.3

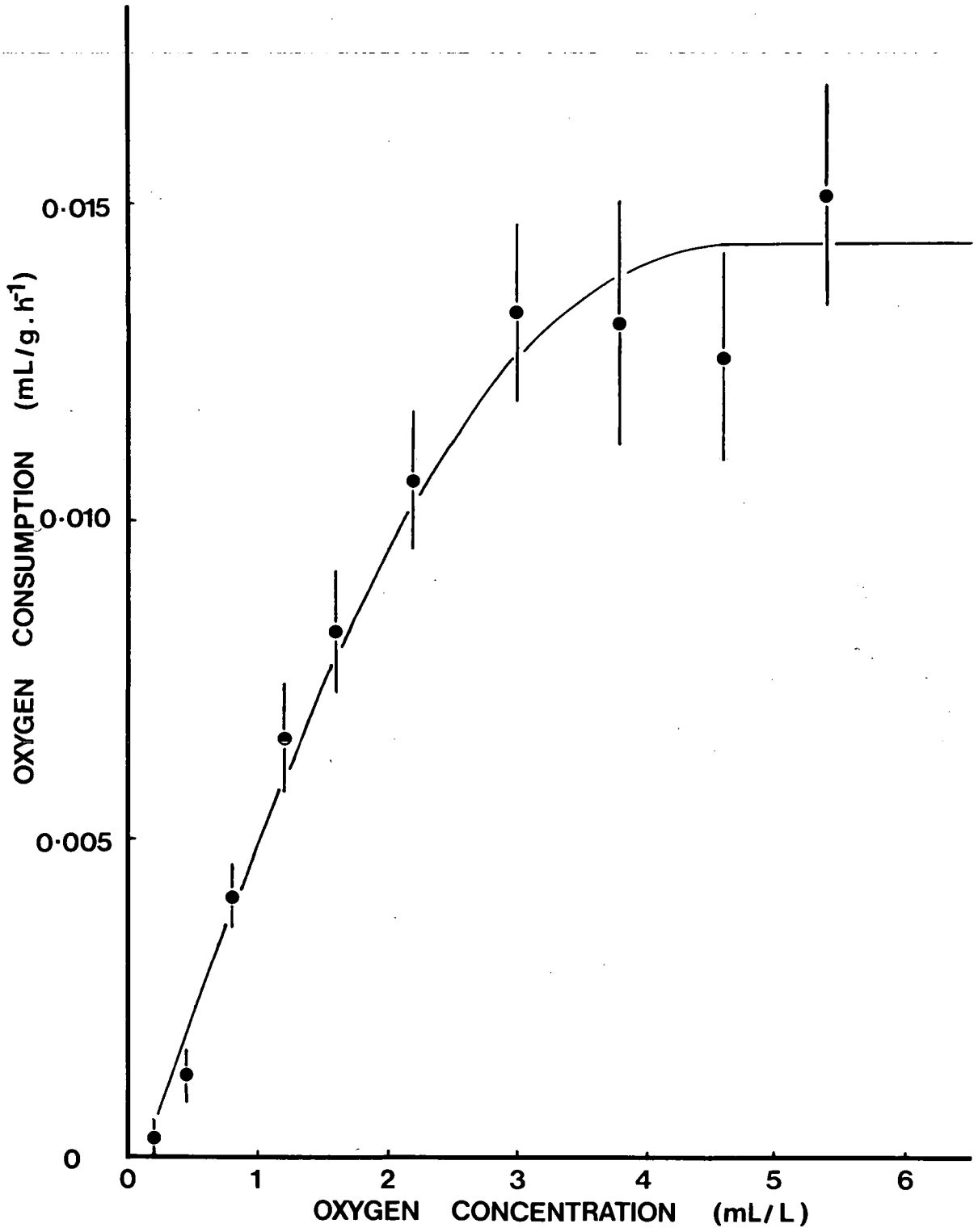

0·015
0·010
0·005
0
OXYGEN CONSUMPTION (mL/g.h⁻¹)
0
1
2
3
4
5
6
OXYGEN CONCENTRATION (mL/L)

Figure 7.4 Relationship between oxygen concentration and oxygen consumption of *Parastacoides tasmanicus*, at 15°C (Mean ± S.E., see Table 7.3 for N values). The curve is the quadratic polynomial which best fits the points, but this equation is only valid to the point of inflexion. Above this point the oxygen consumption is independent of oxygen concentration.

Figure 7.4

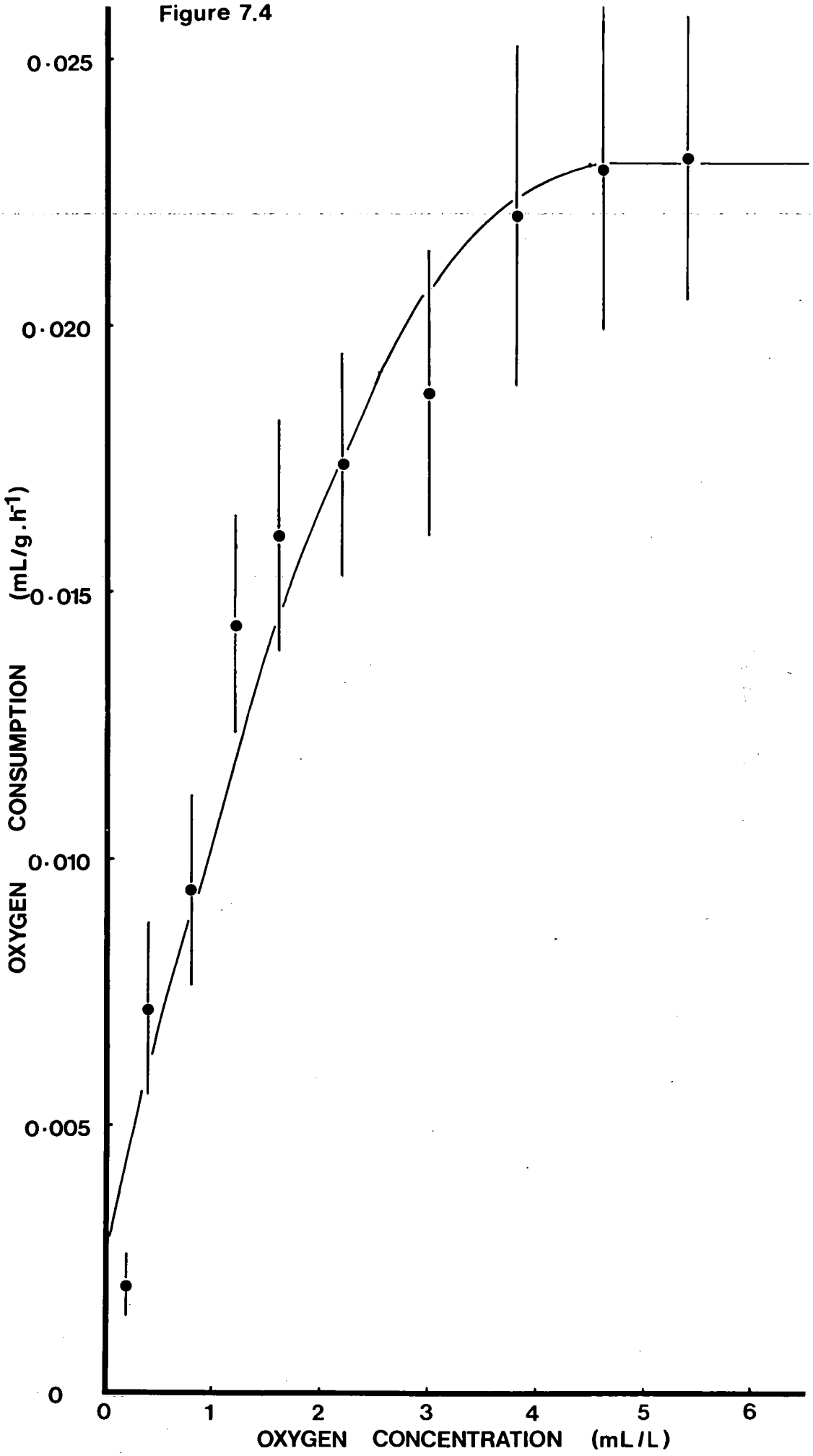

0·025
0·020
0·015
0·010
0·005
0
OXYGEN CONSUMPTION (mL/g.h⁻¹)
0
1
2
3
4
5
6
OXYGEN CONCENTRATION (mL/L)

Table 7.4 Polynomial equations relating oxygen consumption of *Parastacoides tasmanicus* to oxygen concentration.

5°C			
$y = -0.00077 + 0.00660x - 0.00072x^2$			
S.E.	0.00075	0.00072	0.00013
Correlation coefficient 0.925			
N 10			
15°C			
$y = 0.00287 + 0.00887x - 0.00098x^2$			
S.E.	0.00129	0.00124	0.00022
Correlation coefficient 0.915			
N 10			

Note. the 'large' negative values of B_2 in relation to B_1 indicate that *P. tasmanicus* exhibits a considerable amount of respiratory regulation (see van Winkle and Mangum, 1975).

The polynomial equations only satisfy the data when the oxygen concentration is less than about 5.0 mL/L. Above this concentration the oxygen consumption values derived from the polynomial equation decrease with increasing oxygen concentrations, which is definitely not the *real* case. The oxygen consumption is therefore shown as being constant at oxygen concentrations above 5 mL/L in Figures 7.3 and 7.4.

The graphs show that at both 5° and 15°C the oxygen consumption of *P. tasmanicus* is regulated, and independent of external oxygen concentrations at oxygen concentrations above approximately 4.5 mL/L. Below this concentration the oxygen consumption decreases as the oxygen concentration decreases. The 5°C graph shows that oxygen consumption ceases at about 0.15 mL O_2/L, while at 15°C the quadratic regression curve suggests that oxygen consumption still continues when the oxygen concentration is nil. Apparently the quadratic regression curve is unable to adequately cope with the lowest sections of the oxygen consumption curve. However this does not change the fact that *P. tasmanicus* can utilise the

oxygen present even at very low oxygen concentrations.

The incipient limiting tensions at both 5^{o} and $15^{o}C$ are probably somewhat lower than the estimates of 4.5 mL O_2/L that the graphs would suggest. These graphs are actually measurements of routine metabolic rates rather than apparent standard metabolic rates, and the incipient limiting tensions for the routine metabolic rates are probably considerably higher than the incipient limiting tensions for the apparent standard metabolic rates.

The graphs cannot, of course, provide any information concerning the lethal oxygen concentration i.e. the incipient lethal tension.

The experiment on escape responses (see Section 7.2) indicated that at oxygen levels of 0.8 mL/L, and at temperatures of $17^{o}C$ and above, crayfish were not obtaining sufficient oxygen to satisfy their needs, since they left the aquatic medium and commenced aerial respiration. At lower temperatures they made no attempt to leave the water and obviously could obtain enough oxygen. Figures 7.3 and 7.4 show that at $15^{o}C$ animals exposed to an external oxygen concentration of less than 0.8 mL/L would be required to lower their oxygen consumption to less than half the 'normal' rate to survive without extra oxygen, while animals at $5^{o}C$ would have to reduce their oxygen consumption to less than one third of normal. The only way(s) that this could possibly be achieved would be if activity was drastically curtailed, thus bringing about a reduction in oxygen requirements of the animal and/or if some of the required energy was supplied by anaerobic means. The one measurement of lactic acid concentration of an animal that had been exposed to low oxygen concentrations for a considerable period (animal 11 in Table 7.1) suggested that lactic acid is not

accumulated during exposure of an animal to an oxygen concentration of 0.8 mL/L at 15°C (this point will be discussed further in Section 7.12). In addition, if the supply of oxygen in the water was insufficient to meet the crayfish's needs, it would be energetically wasteful to produce lactic acid when it could respire in air.

7.5.1 Aim: To see if *Parastacoides tasmanicus* reduces its activity as a method for reducing its oxygen requirements when in low oxygen conditions.

7.5.2 Materials and methods

The same equipment was used for this investigation as was used in Section 7.2. The sloping floor of the tank was divided into eighths by one line drawn down its middle and three equally spaced lines across it. Activity was measured by scoring '1' each time an animal's chelae crossed a line. Some forms of activity, such as an animal trying to climb out of the container, were assigned arbitrary values according to the intensity of the activity. Activity was scored in 5 minute periods. The scoring was carried out concurrently with the measurements of some of the air/water partitioning of Section 7.2.

7.5.3 Results

Table 7.5 shows the activity of animals in oxygenated and deoxygenated conditions, at 5°, 15° and 17°C. The number codes of the animals are the same as in Section 7.2. The percent of time that animals spent in air in oxygenated and deoxygenated conditions is also included in the results to show whether or not this was related to activity.

At all temperatures tested, the reduction of dissolved oxygen

in the water resulted in a dramatic reduction of an animal's activity. *Prolonged* exposure of an animal to low oxygen tensions resulted in almost complete cessation of its movement. The only exceptions to this generalisation were some of those animals that spent a high percentage of their time out of the water, e.g. animals number 20 and 10, and it is worth noting that although these animals could respire adequately in air they still reduced their activity considerably.

The reduction in locomotory activity is not the only reduction in activity that these animals make in low oxygen conditions. A preliminary experiment showed that the heart rate of crayfish immersed in hypoxic water was also considerably reduced. The total reduction in activity results in a reduction of oxygen demand to the point where the oxygen requirements of *P. tasmanicus* can be satisfied when the oxygen concentration is only 0.8 mL/L (approximately) at 15°C. Even at 17°C some of the smaller animals can obtain enough oxygen at 0.8 mL/L to make it unnecessary for them to leave the water to respire.

Table 7.5 Activity of *Parastacoides tasmanicus* in oxygenated and deoxygenated conditions.

Animal number*	Temp. (°C)	Oxygenated water: Time spent in air (%)	Oxygenated water: Activity† (units/5 min)	Deoxygenated water: Time spent in air (%)	Deoxygenated water: Activity† (units/5 min)
17	17	8.1	6.8 ± 0.8 (6)	0.0	0.8 ± 0.3 (4)
18	17	5.7	21.3 ± 1.2 (6)	95.6	2.2 ± 0.6 (6)
(90-110)	"			88.3	3.0 ± 1.9 (4)
19	17	3.6	18.2 ± 1.2 (6)	83.3	1.6 ± 0.6 (7)
20	17	16.7	46.8 ± 1.0 (6)	56.4	17.4 ± 1.9 (7)
(210-240)	"			62.2	12.4 ± 2.2 (6)
8	15	5.1	38.0 + 9.1 (3)	0.0	18.4 ± 0.5 (5)
9	15	3.9	23.7 ± 3.1 (5)	19.1	2.5 ± 0.3 (6)
10	15	18.3	24.4 ± 2.7 (7)	16.2	13.0 ± 2.1 (7)
(125-180)	"			34.0	7.8 ± 0.6 (11)
11	15	9.8	23.3 ± 2.9 (3)	10.3	5.7 ± 1.4 (10)
(783-843)	"			0.0	3.8 ± 0.9 (12)
12	15	1.7	10.0 ± 0.7 (5)	0.0	0.7 ± 0.3 (3)
(60-105)	"			0.4	2.3 ± 0.6 (9)
(180-200)	"			0.0	0.3 ± 0.3 (4)
(1440-1485)	"			0.0	2.1 ± 0.8 (9)
13	5	14.2	18.5 ± 2.5 (6)	3.6	4.6 ± 1.6 (8)
(1080-1110)	"			0.0	0.0 ± 0.0 (6)
14	5	16.1	12.2 ± 1.3 (6)	10.2	4.7 ± 1.4 (7)
(1320-1350)	"			0.0	0.0 ± 0.0 (6)
(1680-1700)	"			0.0	0.3 ± 0.3 (4)
15	5	10.1	21.8 ± 0.9 (8)	18.5	6.0 ± 0.9 (9)
(190-230)	"			0.0	4.3 ± 0.8 (8)
16	5	23.2	18.3 ± 0.7 (7)	29.4	9.2 ± 1.7 (11)
(115-130)	"			0.0	0.3 ± 0.3 (3)

* Numbers in brackets are minutes after commencement of deoxygenation.
† Mean ± S.E. (N)

7.6.1 Aim: To determine the survival time of *Parastacoides tasmanicus* at very low oxygen concentrations, and the degree of lactic acid accumulation before death.

Parastacoides tasmanicus can survive in low oxygen conditions by reducing its activity and thereby reducing its oxygen requirements, or by leaving the oxygen deficient conditions if the oxygen level is too low. However, in some situations it might not be able to

escape from very low oxygen conditions. How long could it then survive? Would it build up an oxygen debt in the form of accumulated lactic acid, and if so, how much could it tolerate?

7.6.2 Materials and methods

Crayfish were placed in jars (150 -1300 mL capacity depending on the size of the animals used) containing treated button grass water with an oxygen content of approximately 3 mL/L, obtained by bubbling nitrogen through the water for an appropriate period. The jars were closed with perspex lids, each one of which had one small aperture which was closed with a rubber bung, and were placed in the dark at 5^{o} and 15^{o}C. Samples of water (1-2 mL) were taken through the apertures in the lids at regular intervals, and the oxygen concentration of these samples was determined by the micro-Winkler technique. The water that was removed was replaced with water with a very low oxygen content. The survival times of animals were measured from the time the oxygen concentration in the jars dropped below 1 mL/L, and also from the time the oxygen concentration dropped below 0.5 mL/L. At (or preferably just before) the death of each crayfish, its haemolymph lactic acid concentration or total body lactic acid concentration was determined. For measurements of haemolymph lactic acid, haemolymph samples of 0.1 - 0.3 mL were taken from the base of the 5th pair of walking legs and mixed with 1.0 mL of ice-cold 0.6M perchloric acid. The mixture was centrifuged for 10 minutes at 3000 rpm, and 0.2 mL of the supernatant was used to determine the lactic acid concentration with a Boehringer Mannheim GmbH Lactate Test combination. The lactic acid content of whole crayfish was measured as follows. Each crayfish was ground up in a small amount of 0.3M trichloroacetic acid (TCA) in 0.6M hydrochloric acid (after Teal and Carey, 1967). The TCA precipitates proteins and prevents decomposition of the lactic acid, while the hydrochloric acid also helps to stabilise the lactic

acid and decomposes the calcium carbonate in the crayfish exoskeleton. The ground up crayfish was transferred to a 100 mL volumetric flask, and the volume was made up to 100 mL with more 0.3M TCA in 0.6M HCl. The contents of the flask were allowed to stand overnight at 4°C, after which time a sample of the supernatant was taken for measurement of lactic acid content.

The crayfish used in this experiment were not placed directly into anoxic water, as they would then have had no time to acclimate to a low oxygen environment. Their 'normal' oxygen consumption, increased due to stress caused by their having been handled, would have led to a rapid build up of lactic acid before the animals could adjust to their new conditions and consequently a shortened survival time in anoxic conditions. When the crayfish were placed in a moderately low oxygen concentration, they had time to gradually adjust to the onset of hypoxic and then anoxic conditions.

7.6.3 Results

The results of the experiment are presented in Table 7.6, all results as mean ± S.E. (N).

The results show that *P. tasmanicus* can survive for between 62 and 70 hours at 5°C and about 22 hours at 15°C, when the oxygen level is less than 0.5 mL/L, under the conditions imposed in these experiments. During these periods the crayfish builds up lactic acid concentrations of 24·0-25·0 mmol/L in haemolymph and 13·9-18·3 μmol/g wet weight. The total body concentration of lactic acid may be higher in the smaller crayfish as they have a smaller percentage of their body as 'inert' carapace than do larger crayfish.

For the reasons set forth in Section 7.6.2, the crayfish were not placed directly into anoxic conditions, but their survival

Table 7.6 Survival time of *Parastacoides tasmanicus* in hypoxic conditions, and lactic acid concentration at death.

Temp. (°C)	Animal wt. (g)	Lactic acid conc. at death* Haemolymph (mmol/L)	Total body (μmol/g wet wt.)	Survival time Below 1 mL O_2/L (h)	Below 0.5mL O_2/L (h)
5	Control				
	3.4±0.4(4)	2.45±.1.10 (4)	3.66 4.11 4.22	-	-
5	Test				
	0.2±0.0(3)	-	14.40 18.90 21.6	- a	-
	2.5±0.6(4)	-	-	-	69.7± 8.8(4)
	4.1±0.2(7)	24.61±0.61(5)	11.21 16.5	80.4± 5.7(7) b	62.3± 5.0(7)
	5.1±1.5(8)	-	16.5± 2.7 (8)	93.9±23.8(8)	69.1±22.5(8)
15	Control				
	3.8	3.66 (1)	3.6 4.7	-	-
15	Test				
	4.9±0.7(7)	21.31, 21.20	16.1 ±2.9(5)	38.4± 4.0(7)	22.0± 4.1(7)

* Mean ±S.E. (N), or values when N < 4.

a One of these animals excreted 57.3 μmol lactic acid/g body weight while in nearly anoxic conditions.

b Four of these animals excreted 30.1 ±10.2 μmol lactic acid/g body weight.

times in very low oxygen concentrations are probably *equivalent* to about 55 - 60 hours in totally anoxic conditions at 5°C, or about 15-20 hours at 15°C.

The death of the crayfish was not due to an accumulation of waste products in the jars, as even if the water was exchanged for clean water of the same oxygen content halfway through the experiment, the survival time of the crayfish was not affected. Death was also not due to an exhaustion of the carbohydrate reserves of the crayfish. One animal which was near death at 15°C, with a haemolymph lactic acid concentration of 22.2 mmol/L recovered when it was returned to aerated water, and although it was not fed it still managed to build up a lactic acid concentration of 22.2 mmol/L of haemolymph when it was again put in hypoxic conditions a day later. As will be shown later (Section 7.8, 7.9 and 7.10 and also see below), *P. tasmanicus* does not reconvert lactic acid into pyruvate, when it is

returned to normoxic conditions, but rather excretes it; consequently the test animals could not have built up depleted carbohydrate reserves by reconverting lactic acid to glucose.

Measurements of the Na^+, Ca^{++} and K^+ ions in the water in the jars before, during and after the experiments suggested that dying animals lose some of these ions, probably because of tissue damage caused by a build up of lactic acid. Tests were done to see if crayfish excreted any lactic acid during their period in low oxygen conditions. All of these tests were positive, and gave measurements of 57.3 μmol lactic acid/g body weight in one case (a in Table 7.6) and 30.1 ± 10.2 (4) μmol lactic acid/g body weight in another case (b in Table 7.6).

7.7.1 Aim: To determine whether the lactic acid produced during the anaerobic respiration of *P. tasmanicus* is buffered, and the haemolymph ion concentration altered during the accumulation of lactic acid by the crayfish.

It is apparent from the results of Section 7.6 that *P. tasmanicus* can survive with a considerable quantity of lactic acid in its haemolymph and tissues. It is not known if this lactic acid is buffered in any way or whether it affects the ionic content of the haemolymph.

7.7.2 Materials and methods

An initial experiment was performed in which an animal at 5°C was placed in 150 mL of water with a very low oxygen content (oxygen-free nitrogen had been bubbled through the water for 30 minutes) for 46 hours. A haemolymph sample was taken, diluted appropriately and the Na^+, K^+ and Ca^{++} concentrations were measured in an Eel Mk2 flame photometer. Measurements of the ion concentrations in the water in which the animal had been kept were

also taken. Samples were taken from animals which had not been exposed to nearly anoxic conditions for comparison.

7.7.3.1 Results

The results of this experiment are presented in Tables 7.7a and 7.7b.

Table 7.7a Haemolymph-ion concentration of normal crayfish and a crayfish exposed to nearly anoxic conditions for 46 hours at 5°C.

	Weight (g)	Haemolymph ion concentration (mmol/L) Na^+	Ca^{++}	K^+
Control	5.6 7.0	240.0, 247.4	16.7 , 18.0	3.6, 3.6
Test	7.1	309.8	28.7	5.1

Table 7.7b Ion loss from a crayfish exposed to nearly anoxic conditions for 46 hours at 5°C

	Na^+	Ca^{++}	K^+
Ion loss (μmol/46h)	0.055	0.003	0.001

After this preliminary experiment a larger number of animals was subjected to nearly anoxic conditions for 9 hours at 15°C. Haemolymph samples were taken from each of the animals, diluted appropriately and the Na^+ and Ca^{++} concentrations measured. Sodium was measured in the Eel flame photometer while Ca^{++} was measured by the 'glyoxalbis(2-hydroxyanil)' colorimetric method (see Golterman, 1969 for details). Haemolymph from animals not exposed to anoxic conditions was used to obtain control values.

7.7.3.2 Results

The results of this experiment are presented in Table 7.8

Table 7.8 Haemolymph-ion concentration of normal crayfish and crayfish exposed to nearly anoxic conditions for 9 hours at 15°C.

	Weight (g)*	Haemolymph ion concentration (mmol/L)* Na^+	Ca^{++}
Control	5.1 ± 0.5 (4)	242.30 ± 11.26 (4)	16.81 ± 0.77 (4)
Test	5.0 ± 0.4 (5)	225.43 ± 6.43 (5)	27.01 ± 1.82 (5)
t-test	P>0.05	P>0.05	P<0.005

* Mean ± S.E. (N)

When *P. tasmanicus* are in nearly anoxic conditions the Ca^{++} levels in the haemolymph rise significantly, whereas Na^+ (and probably other ions) levels do not. However, this does not mean that Na^+ levels in the body are not affected. Table 7.7b shows that Na^+ was lost from crayfish in nearly anoxic conditions, and as there was no significant change in haemolymph Na^+ concentrations, there must have been a loss of this ion from the tissues. The increase in Ca^{++} levels in the haemolymph is explained by assuming that calcium carbonate was mobilized to buffer the lactic acid as it accumulated, and thereby prevented any marked change in the pH of the haemolymph. Of course this sort of protection is only useful when animals are exposed to anoxic or nearly anoxic conditions for limited periods.

7.8.1 Aim: To determine whether *Parastacoides tasmanicus* repays the 'oxygen debt' it incurs during exposure to anoxic or nearly anoxic conditions, and if so, how long this repayment takes.

As *P. tasmanicus* accumulates lactic acid in very low oxygen conditions, it is important to know how quickly it can dispose of

this 'oxygen debt' when it is returned to oxygenated conditions. The quicker the lactic acid can be removed the less damage it can do, and the quicker the crayfish will be ready to endure another period in very low oxygen conditions.

7.8.2 Materials and Methods

The oxygen consumption of 10 crayfish (6 males, 4 non-berried females) with a weight of 3.1 ± 0.6 (10) g was measured in water, in a Gilson respirometer, at 15°C, over a period of 5 hours. The crayfish were then transferred to airtight containers which contained water with an oxygen content of less than 0.8 mL/L (water through which oxygen-free nitrogen had been bubbled for 30 minutes). The crayfish would have soon exhausted the oxygen remaining in the water in the jars, and would have been in virtually anoxic conditions for the 11 hours they were left in the jars. After this period the crayfish were placed in oxygenated water in vessels on the Gilson respirometer, and after a minimum equilibration time of 15 minutes, the oxygen consumption of each animal was measured. Periodic oxygen measurements were continued for a minimum of 350 to a maximum of 1450 minutes.

7.8.3 Results

The results of this investigation are shown in Figure 7.5, where, because of individual variations in response, the results for each animal are presented separately. In 7 of the 10 graphs, it can be seen that there was an increase in oxygen consumption above the 'initial' rate at some stage during the first 7 hours after removal of the crayfish from anoxic conditions. It was calculated that this excess was only sufficient to repay 0.6 ± 0.3 (7) hours of respiration in anoxic conditions if respiration continued at the equivalent to the 'initial' rate of oxygen consumption during

Figure 7.5 Respiration rates of 10 *Parastacoides tasmanicus* after an 11 hour exposure to almost anoxic conditions. The horizontal line on each graph is the pre-anoxic oxygen consumption rate.

Figure 7.5

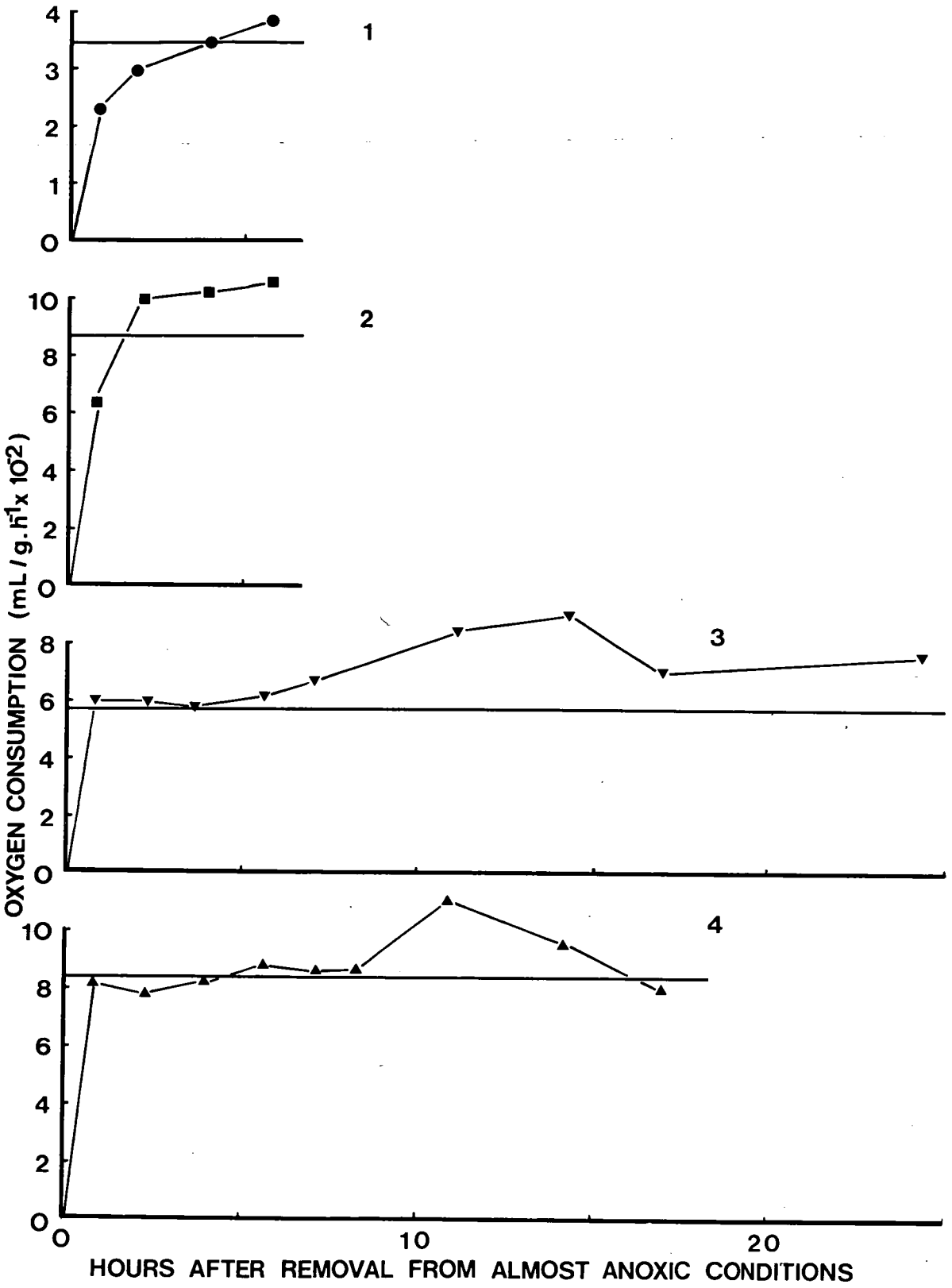

1
2
3
4
OXYGEN CONSUMPTION (mL / g.h^{-1} x 10^{-2})
HOURS AFTER REMOVAL FROM ALMOST ANOXIC CONDITIONS
0
10
20

Figure 7.5 continued

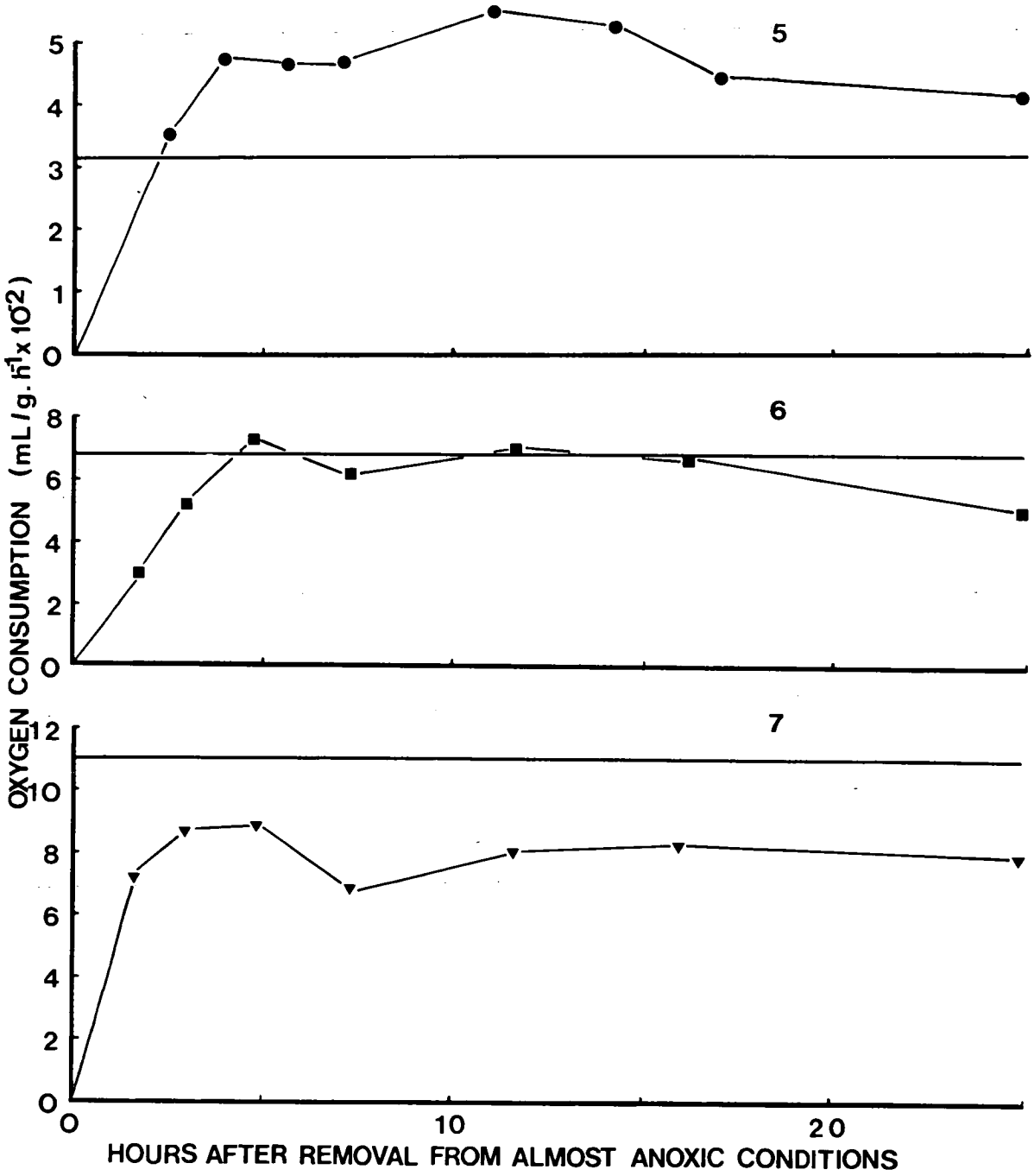
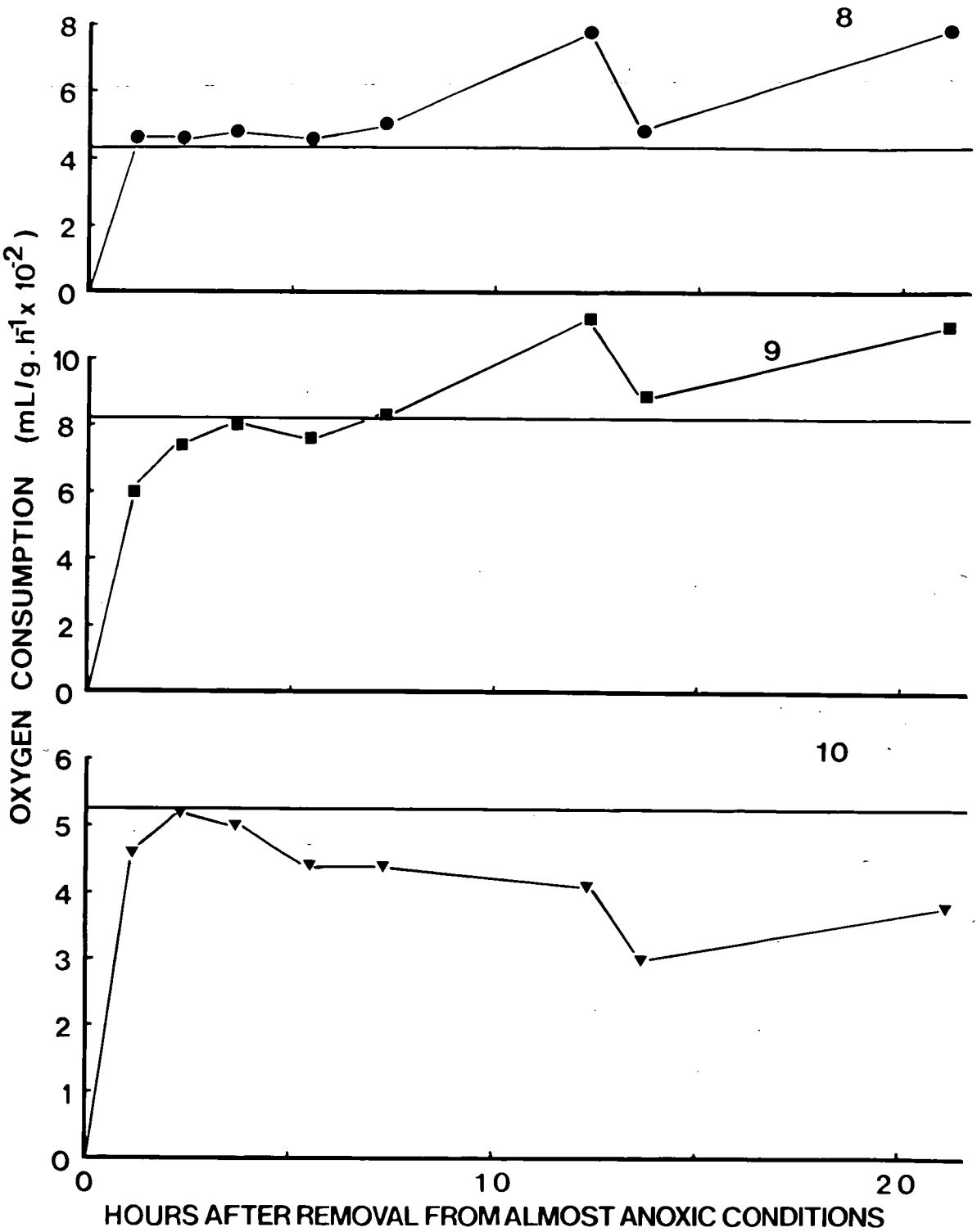


Figure 7.5 continued



the period of anoxia. Even if respiration in anoxic conditions proceeded at a rate equivalent to one-third to one quarter of the 'initial' rate, the increase in post-anoxic conditions would not pay back much of the 'oxygen debt'. In the other 3 graphs, it can be seen that the oxygen consumption during the post-anoxic period is lower than the pre-anoxic rate (for the first 7 hours at least). Three possible alternatives appear to be possible to explain these results:

(1) The oxygen debt is not repaid and the lactic acid is excreted.

(2) The oxygen debt is repaid very slowly and/or only after a considerable delay.

(3) The debt is repaid without increasing the oxygen consumption above pre-anoxic levels.

The last alternative could be possible if the animal were to remain inactive for a period and use only a small proportion of its oxidative metabolism to maintain bodily processes, while using the rest to repay the oxygen debt. To investigate further these possibilities it is necessary that the total body lactic acid concentrations of animals be measured at various times after the removal of the animals from anoxic conditions.

7.9.1 Aim: To measure the rate of removal of lactic acid from the body of *Parastacoides tasmanicus* in aerobic conditions.

7.9.2 Materials and methods

Crayfish of approximately the same size were placed in anaerobic conditions for 9 - 10 hours, at 15^{o}C, and then returned to oxygenated water. Two crayfish were killed every 1 - 2 hours, and their total body lactic acid content was measured by the method given in Section 7.6.2.

Two groups of animals were used. The first, a group of adult

animals with a mean ± S.E. (N) weight of 2.5 ± 0.2 (10) g, was held in anaerobic conditions for 10 hours. The second, a group of juveniles with a mean ± S.E. (N) weight of 0.25 ± 0.03 (10) g, was kept in anaerobic conditions for 9 hours.

7.9.3 Results

The total body lactic acid of animals in the two groups, at various times after their removal from anaerobic conditions, is shown in Figures 7.6.1 and 7.6.2. Figure 7.6.1 shows the lactic acid content of the adult animals following their removal from anaerobic conditions. Lactic acid levels had returned to control levels (4.1 μmol/g wet weight) within 4 hours. Smaller animals (Figure 7.6.2) on the other hand, still had a relatively high level of lactic acid in their bodies after 8 hours in oxygenated water. The lactic acid concentration was only 50% of initial post-anaerobic levels after 6 hours in aerated water though, and the initial level was much higher than it was in the larger animals, probably due to the higher metabolic rate of the smaller animals.

These results preclude the possibility that *P. tasmanicus* repays its oxygen debt slowly. In fact, it strongly suggests that the crayfish does not repay an oxygen debt at all, but rather excretes the lactic acid. Much of the lactic acid disappears during the first hour after removal of animals from anoxic conditions (in large animals at least), and during this period the oxygen consumption of the crayfish is very low.

When *P. tasmanicus* is in anoxic conditions its heart rate, and therefore its circulation are severely reduced. Lactic acid would be expected to gradually build up in the tissues and the haemolymph. If the animal is eventually returned to aerobic conditions the heart rate and circulation rate increase. If the animal is going to excrete the accumulated acid, the most

Figure 7.6.1 Lactic acid content of the bodies of adult *Parastacoides tasmanicus* at different times after their removal from 10 hours exposure to almost anoxic conditions. The horizontal line signifies the normal (control) lactic acid level. Each point on the graph is the mean of values from two crayfish (± range).

If lactic acid concentrations are wanted in mol/g,
the conversion rate is 1 mg lactic acid/g weight = 11.1 μmol/g

Figure 7.6.2 Lactic acid content of the bodies of juvenile *Parastacoides tasmanicus* at different times after their removal from 9 hours exposure to almost anoxic conditions. The horizontal line signifies the normal (control) lactic acid level. Each point on the graph is the mean of values from two crayfish (± range).

Figure 7.6.1

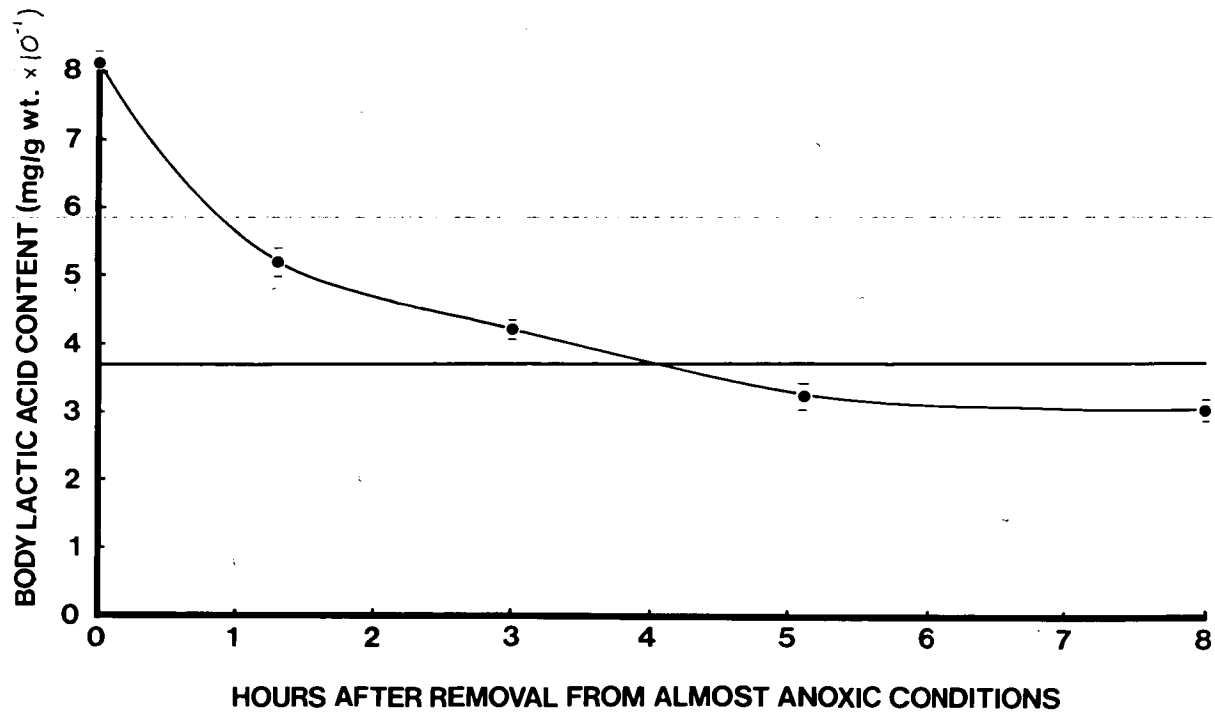
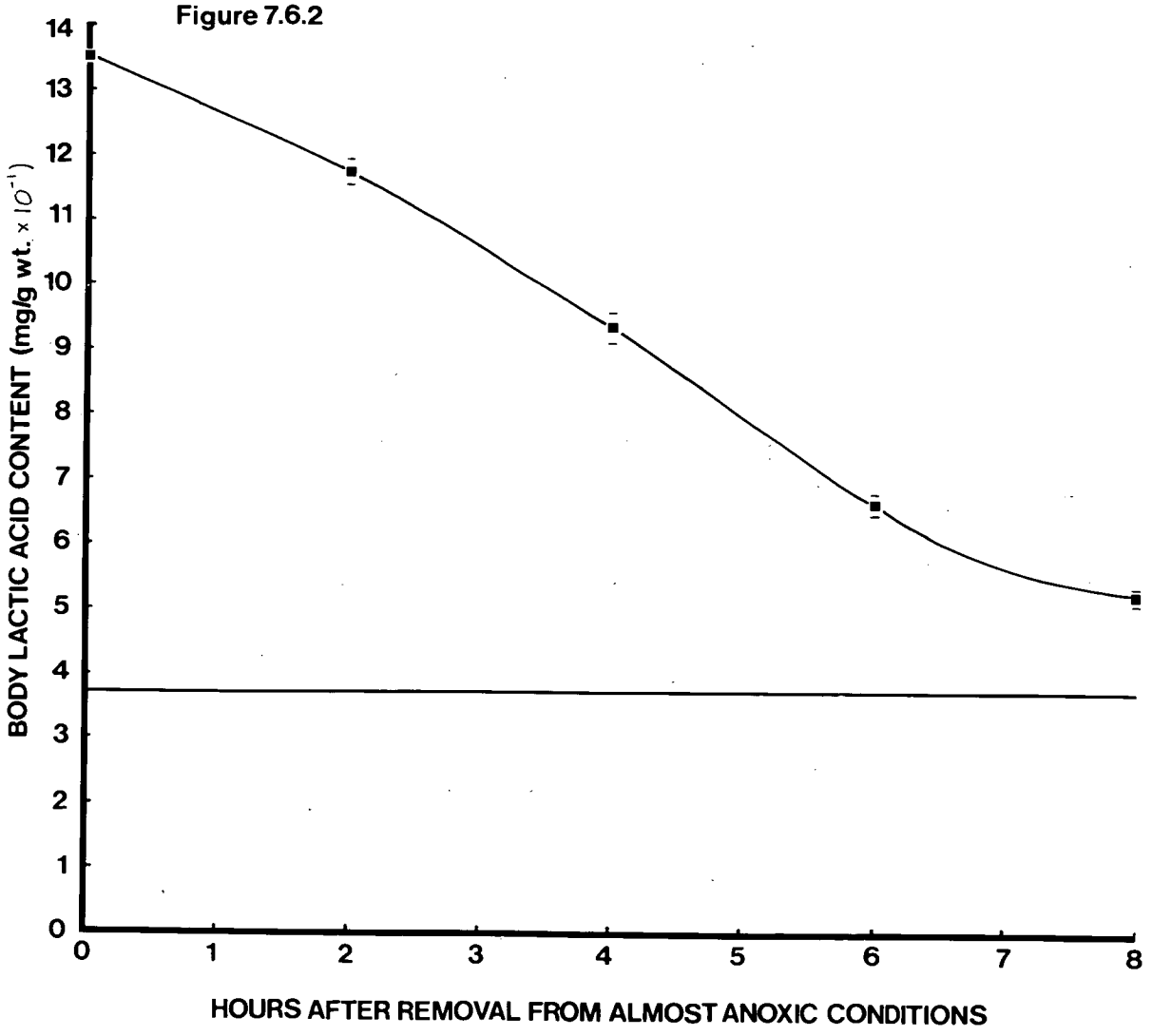


Figure 7.6.2



efficient way, and also the way to minimise tissue damage, would be to rapidly move the lactic acid into the haemolymph, and from there to the gills, where it could be lost by diffusion and/or active transportation. Thus, when an animal is returned to aerated water after a period in anoxic conditions, the lactic acid levels in the haemolymph would be expected to rapidly rise, as the lactic acid is removed from the tissues into the haemolymph. The haemolymph lactic acid level should subsequently start to decrease again as excretion rates exceed the rate of transfer of lactic acid to the haemolymph from the tissues.

7.10.1 Aims: To measure the rate of removal of lactic acid from the haemolymph of *Parastacoides tasmanicus* in aerobic conditions.

7.10.2 Materials and methods

Twelve animals with a mean ± S.E. weight of 5.3 ± 0.2 g were sealed in jars containing water with an initial oxygen content of less than 0.8 mL/L, for 9 hours at 15°C. A further 16 animals with a mean ± S.E. weight of 5.4 ± 0.1 g were kept in nearly anoxic water for 7 hours at 15°C. After the exposure to nearly anoxic conditions the animals were returned to aerated water, and at intervals haemolymph samples were taken from animals (two animals per time) for the measurement of lactic acid content (see Section 7.6.2 for method). From the second group, water samples were also taken from the branchial chamber by means of a 1mL syringe fitted with a 23 gauge needle. The lactic acid concentration of these water samples was also determined.

7.10.3 Results

The graphs which give the lactic acid concentration in the haemolymph of both groups of *P. tasmanicus* at various times after their removal from nearly anoxic water are presented in Figure 7.7. In both groups there is an initial increase in haemolymph lactic acid content, which lasts for 1 - 1½

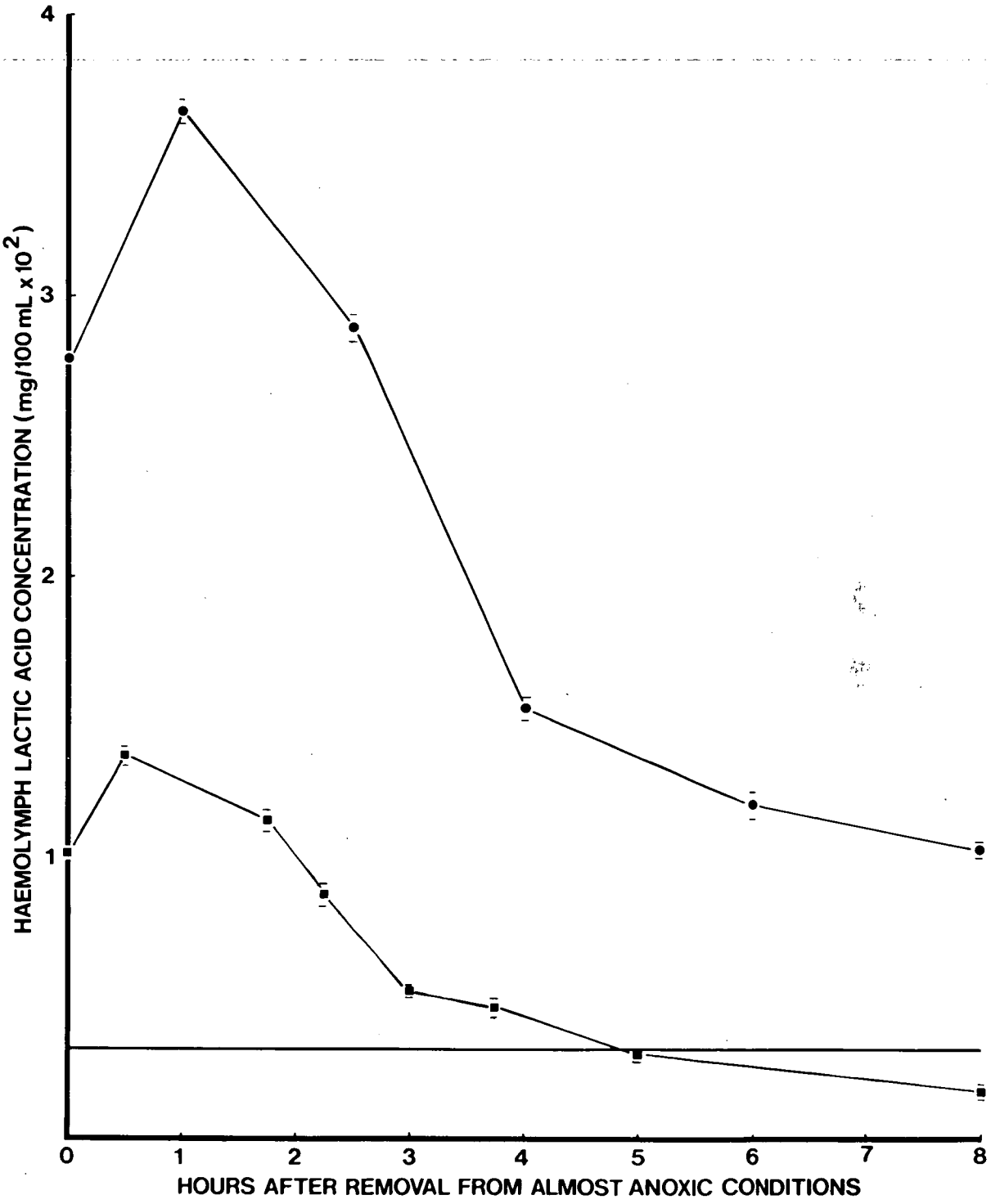
hours. Following this there is a rapid decrease which starts to level out after about 4 hours. The increase in the first 1 - 1½ hours coincides with the initial drop in total body lactic acid (see Figure 7.6.1). The 'flattening out' in the curves in the 4 - 8 hour period occurs after the major levelling off in the total body lactic acid curve has occurred. It is surprising that even after 8 hours the haemolymph lactic acid concentration in the 9 hours exposure group is still fairly high, although the total body lactic acid concentration at this time would be almost at control levels. Similarly, initial values are higher than the values found in the haemolymph of *P. tasmanicus* that had died during exposure to anoxic conditions. The explanation(s) for this is (are) not known although the observation lends circumstantial support to the hypothesis that a rapid transport of lactic acid from the tissues to the haemolymph is an important mechanism for safeguarding tissues.

The lactic acid concentration in the water from the branchial chambers was very variable. This was probably due to the difficulty in obtaining water from a specific area in the branchial cavities. The mean ± S.E. lactic acid concentrations in 7 measurements was 4·78 ± 0·91 mmol/L while that in the water in the containers was less than 0·3 mmol/L, water, which clearly supports the hypothesis that lactic acid is excreted across the gills, although some lactic acid may also be lost via the faeces and/or urine.

The accumulated results show that it is almost certain that *P. tasmanicus* does not pay back an oxygen debt, but rather excretes the lactic acid that builds up during a period in anoxic conditions (when it is returned to oxygenated conditions).

Figure 7.7 Lactic acid concentration in the haemolymph of adult *Parastacoides tasmanicus* at different times after their removal from 7 (■) or 9 (●) hours exposure to almost anoxic conditions. Each point on the graph is the mean of values from two crayfish (± range).
If lactic acid concentrations are wanted in mol/L, the conversion rate is 1mg lactic acid/100 mL = 111μmol/L.

Figure 7.7


HAEMOLYMPH LACTIC ACID CONCENTRATION (mg/100 mL x 10^2)
4
3
2
1
0
1
2
3
4
5
6
7
8
HOURS AFTER REMOVAL FROM ALMOST ANOXIC CONDITIONS

7.11 Summary

Parastacoides tasmanicus copes with low oxygen conditions as follows: At oxygen concentrations down to about 4 mL/L the crayfish regulates its oxygen consumption. Below these oxygen levels, oxygen consumption falls as the oxygen concentration is reduced, but *P. tasmanicus* can reduce its activity and lower its energy requirements so that 'supply' can meet 'demand' even at oxygen levels as low as 0.8 mL/L, at temperatures as high as 15°C. At temperatures above 15°C, or at very low oxygen levels, the crayfish can spend a large proportion of its time respiring in air, and thus may survive indefinitely, even though the water it inhabits does not have enough oxygen to allow it to survive by aquatic aerobic respiration.

If the crayfish is unable to escape from water with a very low oxygen concentration it is then forced to respire anaerobically, and produces lactic acid, while still using all of the oxygen present. It can tolerate several days' accumulation of lactic acid, in anoxic conditions, at 5°C, or almost one day's accumulation at 15°C. The lactic acid is apparently buffered by calcium carbonate (haemolymph pH was not measured but there was a significant increase in calcium content in the haemolymph). If *P. tasmanicus* is returned to aerobic conditions after a period in anoxic (or nearly anoxic) water it soon starts to respire aerobically, but it does not increase its respiration rate in order to metabolize the lactic acid accumulated in the tissues and the haemolymph (Half of the total body lactic acid is in fact in the haemolymph.) Instead, the lactic acid in the tissues is rapidly shunted from the tissues into the haemolymph, which then carries it to the gills, from where it is rapidly excreted (see below). An adult *P. tasmanicus* can return its body lactic acid levels to normal within 4 hours of its removal from a 10 hour exposure to anaerobic conditions.

It is probable that this rapid excretion of lactic acid on return of the crayfish to aerobic conditions is due to an increased circulation efficiency, which accompanies an increased heart rate. Unreported data show that the heart rate of *P. tasmanicus* is directly related to oxygen levels.

7.12 General discussion

Parastacoides tasmanicus has a strategy for survival in low oxygen conditions that is extremely suitable for coping with the problems presented by its environment. The avoidance response is eminently suitable for an animal that lives in a burrow where, especially during warm, dry weather when low oxygen conditions would most commonly occur, part of the burrow is likely to be dry. The only limitation on this behavioural adaptation is the necessity for high humidity levels, but as shown in Chapter 2 this requirement will usually be met.

Parastacoides tasmanicus is not unique in possessing an escape response from poorly oxygenated waters. The crayfish, *Orconectes immunis* (Bovbjerg, 1970), and the crab, *Carcinus maenas* (Taylor and Butler, 1973; Taylor *et al*, 1973; Uglow, 1973) also possess this response. It is not known at what oxygen concentration *Orconectes immunis* leaves the water, but *Carcinus maenas* leaves the water at an oxygen concentration of approximately 2 mL/L at 17°C and 0.8 mL/L at 6°C. *Parastacoides tasmanicus* is more tolerant of hypoxic conditions, and does not leave the water at an oxygen concentration of 0.8 mL/L at 15°C, although at 17°C and the same oxygen concentration, it does.

Parastacoides tasmanicus is a fairly good 'oxyregulator', but its incipient limiting tension, which is about 4 mL O_2/L at both 5° and 15°C is not particularly low. For comparison, *Callianassa californiensis* (Pritchard and Kasparek, 1966; Thompson and Pritchard, 1969) and the *Uca* species studied by Teal and Carey (1967) had incipient limiting tensions of 0.4 - 0.8 mL O_2/L at 10°C, and 1.5 - 2.0 mL O_2/L at 20°C respectively. Even *Homarus gammarus* can regulate its oxygen uptake at oxygen concentrations between 1.4 and 4.9 mL/L (Spoek, 1974). However, the incipient limiting tension

is not really important. What is more important are the observations that *P. tasmanicus* has a low incipient lethal tension (less than 0.8 mL O_2/L at temperatures of 15°C and below), and is able to extract oxygen from the water even at very low oxygen concentrations (about 0.15 mL/L at 5°C and maybe even lower at 15°C). This is comparable to the *Uca* species which can extract oxygen from water with an oxygen concentration as low as 0.1 mL/L at 20°C, and is lower than *Sesarma cinereum* which can only reduce the oxygen concentration to 0.5 mL/L (Teal and Carey, 1967).

The survival of *P. tasmanicus* in totally anoxic conditions is not especially good when it is compared to that of other burrowing Crustacea. *Parastacoides tasmanicus* can only survive the equivalent of about 15 - 20 hours at 15°C and 55 - 60 hours at 5°C in anoxic conditions, compared to at least 24 hours for *Uca pugnax* at 21°C (Teal and Carey, 1967), and three days for *Callianassa californiensis* and *Upogebia pugettensis* at 10°C (Thompson and Pritchard, 1969). However, *P. tasmanicus* is more likely to have to cope with long periods of hypoxic conditions rather than with periods of total anoxia, and this it is well adapted to do.

The apparently low 'incipient lethal tension' of *P. tasmanicus* may be based on a combination of two factors. Firstly, *P. tasmanicus* has a low metabolic rate which can be reduced further by a cessation of all but the most vital activities, and sufficient oxygen can be obtained to satisfy these needs at very low oxygen concentrations. Secondly, even if the oxygen concentration is so low that all the required oxygen cannot be obtained, the deficit can be made up by anaerobic respiration, with excretion of the lactic acid produced. As no toxic products are accumulated, the incipient lethal tension is lower than the lowest oxygen concentration at which *P. tasmanicus* can satisfy its energy requirements by aerobic means.

In totally anoxic conditions lactic acid does accumulate in *P. tasmanicus*, eventually resulting in the animal's death. *Parastacoides tasmanicus* can tolerate a maximum of 21.0-25.0 mmol lactic acid/L of haemolymph or 13.9 - 18.3 µmol lactic acid/g weight. This is fairly low when compared to *U. pugnax* which has been found to accumulate up to 80 µmol lactic acid/g weight in anoxic conditions at 20°C (Teal and Carey, 1967) or *Upogebia pugettensis* which accumulates 41.7 ± 3.6 mmol lactic acid/L haemolymph in 18 - 20 hours in anoxic conditions at 10 - 13°C (and it can survive anoxic conditions for *3 days*) (Pritchard and Eddy, 1979). Once again this is not very important, as *P. tasmanicus* does not usually find itself in totally anoxic conditions, but in conditions where the oxygen level is just very low. In these conditions *P. tasmanicus* can excrete the lactic acid that it produces. Thus, when *P. tasmanicus* is unable or unwilling to leave low oxygen conditions it would be able to satisfy its energy requirements with aerobically and anaerobically produced energy. If the oxygen tension became even lower, more lactic acid would be produced, as the aerobic energy supply was reduced, and at a low enough oxygen level the lactic acid would be produced faster than it could be excreted, and would start to accumulate. At very low oxygen tensions there is the added problem that circulation rate would be so reduced that lactic acid would be excreted much more slowly than in aerobic conditions. However, as long as the oxygen level was not too low, *P. tasmanicus* could accumulate lactic acid for several weeks before critical concentrations were reached. Eventually either the oxygen level would increase sufficiently (although still remaining too low to enable the animal to metabolise the lactic acid) so that excretion of lactic acid would exceed its production rate, or else the animal would be able to leave the water and respire in air. If the

former is the case the crayfish would soon be ready to endure another period of low oxygen levels, but in the latter case it is unlikely that it could excrete the lactic acid very fast, so it might then have to metabolise the lactic acid.

The excretion of lactic acid by *P. tasmanicus*, rather than being wasteful, is probably a major factor in this animal's ability to survive the low oxygen tensions that often occur on the button grass plains.

The exposure to chronic low oxygen conditions has elicited a different resonse from *P. tasmanicus* than that shown by some intertidal bivalves, for example. These animals endure regular and rhythmic exposure to anoxic conditions followed by exposure to well oxygenated water, as occurs when they are exposed to air at low tide and keep their valves shut, and then are covered by oxygenated water when the tide comes in. Some of these animals respire anaerobically during the exposure to anoxic conditions (while buffering the acid products with calcium carbonate) and repay the oxygen debt that they incur, during the aerobic periods (Akberali *et al*, 1977; Bayne *et al*, 1976). Similarly, the crab, *Uca pugnax*, which can accumulate large quantities of lactic acid, and which subsequently repays this oxygen debt, is unlikely to be exposed to anoxic conditions for too long. It begins anaerobic respiration when the oxygen tension in its burrow falls during high tide, but it can oxidise the accumulated lactic acid at the next low tide when it is back in air. After a period in anoxic conditions *U. pugnax* needs to be returned to aerobic conditions where is can increase its oxygen consumption to a level where it can resynthesize the lactic acid into a more useful and less toxic form. If it was exposed to chronic hypoxic conditions as *P. tasmanicus* sometimes is, it could not remove its lactic acid

and would eventually accumulate enough to kill it, whereas *P. tasmanicus* can excrete the lactic acid that it produces if the oxygen tension is not too low, for too long.

8. GENERAL CONCLUSIONS

Several of the preceding chapters are concerned with the physiological adaptations exhibited by *Parastacoides tasmanicus* to what are believed to be the important physical factors in its environment. Thus, investigations into the effects of pH are reported in Chapter 3, whilst Chapter 4 is concerned with the responses shown in the absence of burrow water, and Chapter 7 is a report of a study of the responses to chronic low oxygen conditions. In all these chapters the influence of temperature is also examined. These studies show that the animal is well adapted to its environment and is capable of coping with the whole range of expected environmental conditions. In addition, it has been shown that *P. tasmanicus* has a digestive system that enables it to efficiently utilise the food available in its environment (Chapter 5). Examination of the changes in tissue composition with seasons and moult stages indicates that mature *P. tasmanicus* females only breed every second year, producing a relatively small number of large eggs. However, *P. tasmanicus* probably lives longer than other freshwater crayfish, and this longevity compensates for the low reproductive capacity (Chapter 6).

The methods used by *P. tasmanicus* to cope with various aspects of its environment may not at first sight appear to be related. However, closer examination reveals that in its adaptation to its environment this crayfish has used several mechanisms for multiple purposes, and some of these mechanisms affect a number of aspects of its life. This final section of the thesis attempts to review these mechanisms and place them into an overall context.

The integument of *P. tasmanicus* appears to be relatively impermeable to diffusion of water (Chapter 4). Work presented in Chapter 3 also indicates that *P. tasmanicus* is likely to be

relatively impermeable to passive diffusion of ions (at least to H^+ and OH^- ions). Low permeability to ions and water would be expected to serve a number of purposes. Firstly, reduced permeability to ions would result in lower loss rates for Ca^{++} and Na^+ ions than in other freshwater crayfish, and this would be an advantage to an animal living in an ion-deficient environment. Almost as important in the habitat occupied by *P. tasmanicus* would be the fact that entry of H^+ (and OH^-) ions would also be restricted. Low permeability to ions would therefore play a major part in allowing the crayfish to maintain a haemolymph pH within the normal range found for other invertebrates from less extreme habitats, and to survive in a very wide range of external pH without any obvious signs of stress. The low permeability to water also presumably confers several advantages on the crayfish. The most important of these is likely to be consequent upon the low rate of influx of water. This would be expected to result in only small volumes of urine being required, so urinary loss of ions should be minimal. Secondly, when the crayfish is removed from water, its low permeability to water means that it has a low rate of evaporative water loss and will not rapidly dehydrate in a 'dry' burrow, or on nocturnal excursions across the button grass plains.

In summary then, adaptations which may be considered advantageous in an ion-deficient environment, also allow *P. tasmanicus* to survive in a very wide range of pH and to endure periods without free water. This does not imply, of course, that all animals living in an ion-poor environment will be tolerant of a wide range of pH, as the method of adaptation may be different in different species.

If low permeability to water and ions is a result of some physical barrier, caused by a change in the structure of the integument (for example), then it may be that the rate of diffusion of oxygen

across the gills of *P. tasmanicus* is also low, and this may have been the origin of the low oxygen consumption of the crayfish. Information on the haemolymph PO_2 and haemocyanin-oxygen equilibrium curves would be necessary before this possibility could be considered, and unfortunately this data is not available. Whatever the origin of the low VO_2 of *P. tasmanicus*, it is obvious that this crayfish is *now* adapted to living in water with a low oxygen content, as it can extract oxygen from water even at a very low external PO_2, and has a reasonably low incipient lethal tension. These observations would appear to be inconsistent with the need for a large PO_2 gradient to drive oxygen across a poorly permeable gill epithelium.

Whilst the low oxygen consumption of *P. tasmanicus* compared to other decapod crustaceans facilitates survival in poorly oxygenated waters, it would appear to have important consequences as far as the animal's life cycle is concerned. When coupled with the fact that *P. tasmanicus* does not compensate for seasonal temperature changes, the low oxygen consumption leads to a metabolic rate in winter so low that very little in the way of searching for food, growth or accumulation of storage products can take place, and so development of ovaries and storage of materials in the midgut gland of females (for example) is restricted to the warmer months of the year. This restriction, together with the long period for which the eggs are carried, which in turn restricts moulting and egg production to a 2 to 3 month period, is responsible for the two year reproductive cycle exhibited by this species. However, the low metabolic rate might also account for the suggested long life span of *P. tasmanicus* (in comparison to the short life time of, for example, many North American crayfish) and this longevity is likely to compensate for the longer breeding cycle.

There is also a further reason why the growth of *P. tasmanicus* during winter is limited. During winter the activity of at least some of the digestive enzymes will be lower than in summer (see Chapter 5) because of the lower environmental temperatures, and although assimilation efficiencies are not reduced since food takes longer to pass through the digestive tract at the lower temperatures, this lower rate of passage must in turn reduce the amount of food that can be eaten and so reduce nutrient uptake and subsequent growth. The higher temperatures obtained during summer will result in increased enzymatic activity, more rapid movement of food through the digestive tract, and an increased net uptake of nutrients, so that growth will be faster. During warmer months the availability of high protein food, in the form of insects and other small animals, is also likely to be higher than during winter.

The ability of *P. tasmanicus* to respire in air is another ability which serves several purposes. The crayfish can use this ability both to survive when the available burrow water has a low oxygen tension, and when there is no free water at all and the animal must survive in a 'dry' burrow. From the crayfish's point of view the problem is compounded because these conditions are likely to arise in summer when activity is greatest and oxygen demands are at their peak. If it were not able to respire adequately out of water the crayfish would be restricted to permanently wet areas in which oxygen levels remained relatively high throughout the year; this would make much of its present range uninhabitable. It is likely that *P. tasmanicus inermis* (not studied in this thesis) is exploiting this ability to respire in air even more than *P. tasmanicus tasmanicus* or *P. tasmanicus insignis* since it lives in drier areas than the other sub-species. It would be interesting to know if its rate of aerial respiration is increased, and also

whether there is any reduction in its ability to respire in poorly oxygenated conditions.

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